Novel steroidal inhibitors of HIV-1 protease

This item was submitted to Loughborough University's Institutional Repository by the/an author.

Additional Information:

- A Doctoral Thesis. Submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy of Loughborough University.

Metadata Record: https://dspace.lboro.ac.uk/2134/14701

Publisher: © James Jonathan Härburn

Please cite the published version.
This item was submitted to Loughborough University as a PhD thesis by the author and is made available in the Institutional Repository (https://dspace.lboro.ac.uk/) under the following Creative Commons Licence conditions.

You are free:

- to copy, distribute, display, and perform the work

Under the following conditions:

**Attribution.** You must attribute the work in the manner specified by the author or licensor.

**Noncommercial.** You may not use this work for commercial purposes.

**No Derivative Works.** You may not alter, transform, or build upon this work.

- For any reuse or distribution, you must make clear to others the license terms of this work.
- Any of these conditions can be waived if you get permission from the copyright holder.

Your fair use and other rights are in no way affected by the above.

This is a human-readable summary of the Legal Code (the full license).

Disclaimer

For the full text of this licence, please go to:
http://creativecommons.org/licenses/by-nc-nd/2.5/
Novel Steroidal Inhibitors of HIV-1 Protease.

By

James Jonathan Harburn

A Doctoral Thesis
Submitted in partial fulfillment of the requirements for the award of

PhD Degree of Loughborough University

June 1998

© James Jonathan Harburn, 1998
Acknowledgments.

This thesis is dedicated to mum, dad, Andrea and family for their support both mentally and financially during my stay at Loughborough. Thankyou for everything you have done for me.

I would like to express my sincere thanks to Professor Brian Marples for his help, encouragement, supervision and above all patience during my research.

I would also like to thank Loughborough University for the Postgraduate Research Studentship and the Medical Research Council for the biological testing of the drug candidates.

I would like to take this opportunity to thank the technical staff at Loughborough University Chemistry Department: Alistair Daley (GC-MS), John Kershaw (NMR and MS), John Spray (glass blowing), EPSRC Mass Spectroscopy Unit, Swansea University and a special thanks to Dr. Tim Smith (NMR).

I would like to thank Dr. Steve Christie and Dr. Dave Riddick (Cambridge) for their help and advice concerning molecular modeling studies.

Thanks goes to past and present members of the Chemistry Department. Special thanks goes to the two people who have helped and inspired me to go further with my chemistry research career, namely Dr. Todd Boehlow and Dr. Tim Smith.

I would like to thank all of the residents, past and present of ‘The Palace’ and ‘101 Albert Promenade’ for their amusement and merriment.

Finally, I would like to express my thanks to the many people who have kept me sane (to a degree) over my time at Loughborough: Eb, Damo, Simon, ‘Jitsu’ Phil, Alistair, Captain Miller, ‘Fat now thin’ Rich, Faz, Rob, Chester and everyone associated with the Jitsu club.
CONTENTS

Acknowledgments. (i)

Contents. (ii-iii)

Abbreviations. (iv)

Abstract. (v)

CHAPTER 1.

1.1 INTRODUCTION. 2

1.2 HIV and AIDS. 3

1.3 AIDS Types. 4

1.4 Epidemiology and Spread of HIV-1. 6

1.5 Structure of HIV-1.
   1.5.1 The Viral Envelope. 7
   1.5.2 The Viral Core. 8

1.6 The Replication of HIV-1.
   1.6.1 Entry into the Human Cell. 10
   1.6.2 Producing and Inserting the Viral DNA. 10
   1.6.3 From Provirus to mRNA. 11
   1.6.4 The Role of Protease in the Maturation of the Virus. 11
   1.6.5 HIV-1 Protease Inhibition. 12

1.7 Structural Properties of HIV-1 Protease. 13

1.8 Design of Protease Inhibitors.
   1.8.1 Peptide Derived Inhibitors. 15
   1.8.2 Non-Peptide Derived Inhibitors. 16
   1.8.3 Combination of Protease and Reverse Transcriptase Inhibitors. 26

1.9 Steroids. 32

1.10 Design of Steroidal Inhibitors of HIV-1 Protease. 36
Abbreviations.

AIDS Acquired Immune Deficiency Syndrome
HIV Human Immunodeficiency Virus
SIV Simian Immunodeficiency Virus
RNA Ribonucleic Acid
DNA Deoxyribonucleic Acid
Amp 2-(aminomethyl)pyridine
Ile Isoleucine
Noa (1-napthlyoxy)acetyl
CIC\textsubscript{95} Critical Inhibitory Concentration at which point 95% of the virus is inactive
PBL, MT4, H9, CEM-T4 Different biological cell lines
TBDMSCI tert-butyldimethylsilyl chloride
DEAD diethylazodicarboxylate
DBAD di-\textit{tert}-butylazodicarboxylate
NIS N-iodosuccinamidene
EEDQ N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
DMAP 4-Dimethylaminopyridine
PCC Pyridinium chlorochromate
nOe nuclear Overhauser effect
Boc tert-butoxycarbonyl
9-BBN 9-borabicyclo[3.3.1]nonane
LDA lithium di-\textit{iso}-propylamide
LHMDS lithium hexamethyldisilazide
DMPU 1,3-dimethyl-3,4,5,6-tetrahydro-2-pyrimidone
\textit{m}CPBA \textit{meta}-chloroperbenzoic acid
DDQ dicyanodichloroquinone
BSA benzene seleninic anhydride
XTT 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino)carbonyl]-2H-tetrazolinium hydroxide
Abstract.

Novel Steroidal Inhibitors of HIV-1 Protease.

This thesis describes the synthetic routes investigated in order to prepare 11-amino-12-hydroxy steroids (bile acids and estrogens) and substituted derivatives as potential competitive inhibitors.

Methyl 11-amino-3α-benzyloxy-12-oxo-5β-chol-9(11)-en-24-oate was prepared from deoxycholic acid in five steps by the formation of key intermediate methyl 3α-benzyloxy-11α-bromo-12-oxo-5β-cholan-24-oate. SN2 displacement of bromine with azide and subsequent base-catalysed elimination of nitrogen leads to the enamino-ketone.

Pivaloyl, Cbz and Ethoxycarbonyl amino protected derivatives were synthesised from the enamine and all four compounds were tested for HIV-1 protease inhibitory activity by the Medical Research Council. All four compounds tested showed modest inhibitory activity, the most active being the pivaloyl derivative as predicted previously by molecular modeling.

Introduction of stereocentres at C-11 and C-12 by reduction of Δ9(11) and / or 12-ketone proved unsuccessful.

An alternative route to 11-amino-12-hydroxy steroids was attempted through nucleophilic ring opening of methyl 3α-benzyloxy-11α,12α-epoxy-5β-cholan-24-oate with a range of nitrogen nucleophiles in both the presence and absence of Lewis acids. Ring opening was achieved but without retention of the 11-nitrogen 12-oxygen system.

Routes to C-ring functionalised estrogens from bile acids have also been investigated. A number of A-ring 1,4-dien-3-ones (Δ9(11)-12-one, 12-one, Δ11 and 11α,12α-epoxy derivatives) have been prepared and attempts at aromatisation of the A-ring have been investigated. The products formed depend upon the position and type of C-ring substituents and provide new evidence in support of a radical anion mechanism when reacted with activated zinc in wet pyridine.
Chapter 1.
1.1 INTRODUCTION.

In the last decade, the rapid emergence of the Acquired Immune Deficiency Syndrome (AIDS) as a health threat of global proportions has instigated extensive research of it's etiological agent, the Human Immunodeficiency Virus (HIV),¹ in order to develop effective therapies. Worldwide, an estimated 27.9 million people had become HIV-infected through mid-1996 and 7.7 million had developed AIDS according to the World Health Organisation (WHO). Various projections indicate that by the year 2000, between 40 and 110 million people worldwide will be HIV-infected.

Since it's discovery in the mid-1980's, the current understanding of the expression and function of the various genes of HIV, the life cycle in infected blood cells and pathological course in infected humans and animals has very quickly reached a high level of sophistication.

From this, several targets of the gene products of HIV, especially reverse transcriptase (RT), integrase (IN) and protease (PR) have become popular targets for chemotherapeutic intervention, for which inhibitors or inactivators have been discovered and investigated to variable extents as potential drugs.

The basic hypothesis that specific anti-retroviral agents should ameliorate the course of HIV infection is now supported by clinical trials with nucleoside-based inhibitors of the viral reverse transcriptase² and in combination therapy with inhibitors of both viral reverse transcriptase and protease.³

The nature and pathological consequences of the HIV infection provide remarkable challenges for the progress of effective therapies for the treatment of AIDS.
1.2 HIV and AIDS.

The HIV disease is characterised by a gradual deterioration of the immune function. Most notably, crucial immune cells called CD4+ T cells are disabled and killed during the typical course of infection. These cells, sometimes called 'T-helper cells', play a central role in the immune response, signalling to other cells in the immune system to perform their special functions. A healthy, uninfected person usually has 800 - 1200 CD4+ T cells per cubic millimetre (mm$^3$) of blood. During HIV infection, the number of these cells in a person's blood progressively declines. When a person's CD4+ T cell count falls below 200 / mm$^3$, he or she becomes particularly vulnerable to the infections and cancers that typify AIDS, the end stage of HIV disease. People with AIDS often suffer infections of the intestinal tract, lungs, brain, eyes and other organs, as well as debilitating weight loss, diarrhoea, neurological conditions and cancers such as Kaposi's sarcoma (connective tissue tumour) and lymphomas (lymphatic system tumour).

Most scientists think that HIV causes AIDS directly by killing CD4+ T cells and by triggering other events that weaken a person's immune function. For example, the network of signalling molecules that normally regulates an immune response is disrupted during HIV disease, impairing a person's ability to fight other infections. The HIV-mediated destruction of the lymph nodes and related immunological organs also plays a major role in causing the immunosuppression seen in people with AIDS.
1.3 AIDS Types.

There are, in fact, two human AIDS viruses; HIV-1 and HIV-2. When the 'AIDS virus' is referred to in the media, the most prevalent, HIV-1, is being talked about, but the differentiation between the two must be made.

![Family Tree of Primate Lentiviruses](image)

**Fig. 1** - Family Tree of Primate Lentiviruses ('Long-incubation' viruses).

Fig. 1 above shows a family tree of human and monkey AIDS viruses. It is based upon analysis of the genetic information of the viruses, and shows that HIV-2 is basically identical to a group of monkey AIDS viruses from sooty mangabey monkeys (SIV$_{smm}$). Also, from Fig. 1 it can be seen that HIV-1 is very similar to SIV$_{cpz}$, a chimpanzee virus.

This means that, most likely, HIV-2 started out in sooty mangabey monkeys and at some point it entered human beings, where it evolved, adapted and became HIV-2. This happened in the fairly recent past (a few hundred years, at most) since HIV-2 and SIV$_{smm}$ are still very similar and have not yet diverged appreciably.
It is important to realise that SIVsmm does not cause disease in its natural host (sooty mangabey monkey) whereas HIV-2 does cause disease in humans, implying that viruses and their hosts often co-evolve (biologically, it is not acceptable if a virus kills its own host, as it is difficult for the virus to reproduce itself and consequently, difficult for the infection of new hosts).

One prediction of this theory of virus evolution is that, in time, one might expect HIV-1 to become less lethal in humans due to the virus being able to adapt to a human host. However, there is no evidence to support such a statement. Other things worthy of note concerning HIV-2 are that:

There is no HIV-2 pandemic - that is, there is no global epidemic of HIV-2. In fact, HIV-2 is still mainly restricted to West Africa (eg, Cameroon, Ivory Coast, Senegal). In comparison to HIV-1 this is completely different, suggesting that HIV-2 may not be transmitted as efficiently as HIV-1. Current studies are underway to understand the reasons why.

HIV-2 appears to be less pathogenic than HIV-1. HIV-2 incubation times are much longer than that of HIV-1. Again studies are underway to understand this anomaly.
1.4 Epidemiology and Spread of HIV-1.

The current global HIV-1 epidemic is shown in Fig. 2.

As Fig. 2 indicates, the largest number of HIV-1 infected persons is in sub-Saharan Africa. However, the epidemic is actually growing the fastest in South-East Asia (countries such as India and Thailand).

Fig. 2 also shows some letters (A through to G); these refer to the different subtypes of the HIV virus that are present in different parts of the world. As can be seen, subtype B is most prevalent in Western Europe and in the USA, whereas subtypes E and C are more common in Southeast Asia.

Different subtypes of HIV-1 are important in deciding what kind of vaccine or drug to use with respect to the area of the world.

There is some evidence to suggest that HIV-1E may be more efficient at infecting people by a heterosexual route, compared to HIV-1B.6
1.5 The Structure of HIV-1.

Shown in Fig. 3, is the spherical HIV-1 virus particle (diameter approximately 1100 Å) and comprises two portions; an envelope and a core.

1.5.1 The Viral Envelope.

The outer coat of the virus, known as the viral envelope, is composed of two layers of fatty molecules called lipids, taken from the membrane of a human cell when a newly formed virus particle buds from the cell. Embedded in the viral envelope are proteins from the host cell, as well as 72 copies (on average) of a complex HIV protein that protrudes from the envelope surface. This protein known as env, consists of:

- the transmembrane protein (TM, gp41, Fig. 3), a stem consisting of three or four molecules that anchor the structure in the viral envelope;
- the knob-like surface protein (SU, gp120, Fig. 3) on the outside made up of three or four molecules;
- and the matrix protein (MA, p17, Fig. 3) on the inside.

The abbreviation for the protein (p) or glycoprotein (gp) is followed by a number that gives the approximate molecular size of the protein in
kilodaltons as determined by electrophoresis. Due to the experimental error associated with this technique, slightly different molecular weights may appear in the literature for the same substance. Much of the research to develop a vaccine against HIV has focused on these envelope proteins.

1.5.2 The Viral Core.

The core portion is roughly bullet-shaped, with an outer ‘skin’ formed from 2000 copies of the capsid protein (CA, p24, Fig. 3). The capsid surrounds two single strands of HIV RNA (Ribonucleic acid), each of which contains a copy of the virus’ nine genes. Three of these genes, gag, pol and env (Fig. 4), contain the information needed to make structural proteins for new virus particles. The env gene, for example, codes a protein called gp160 that is broken down by a viral enzyme to form gp120 (SU, Fig. 3) and gp41 (TM, Fig. 3), the components of env.

Three regulatory genes, tat, rev and nef, and three auxiliary genes vif, vpr and vpu, contain the information necessary for the production of proteins that control the ability of HIV to infect a cell, produce new copies of virus or cause disease (Fig. 4). The protein encoded by nef, for instance, appears necessary for the virus to replicate efficiently and the vpu-encoded protein influences the release of new virus particles from infected cells.

The ends of each strand of HIV RNA contain an RNA sequence called the long terminal repeat (LTR). Regions in the LTR act as switches to control the production of new viruses and can be triggered by proteins from either HIV or the host cell. Each of the viral RNA strands are tightly covered by the nucleocapsid protein (NC, p7, Fig. 3). Three enzymes are associated with the RNA-NC complex:

- protease (PR, p11, Fig. 3);
- reverse transcriptase (RT, p66, Fig. 3);
- and integrase (IN, p32, Fig. 3).
Another protein called p17, or the HIV matrix protein, lies between the viral core and the viral envelope. The virus also contains a protein of unidentified function, designated p6.

HIV belongs to a class of viruses called retroviruses, which have genes composed of ribonucleic acid (RNA) molecules. The genes of humans and most other organisms are made up of a related molecule, deoxyribonucleic acid (DNA). Like all other viruses, HIV-1 can replicate only inside cells, commandeering the cell's machinery to reproduce. However, only HIV-1 and other retroviruses, once inside a cell, use the enzyme reverse transcriptase to convert their RNA into DNA, which can be incorporated into the host cell's genes before the usual process of producing protein can occur (Fig. 5).
1.6 The Replication of HIV-1.

To understand the function of the protease, one must become familiar with the replication of the virus, a process that can be best discussed when separated into stages.

1.6.1 Entry into the Human Cell\(^1\) (Fig. 6, \(^\circ\)).

![Diagram of HIV replication](image)

**Fig. 6 - Replication of HIV-1.**

When the virus attacks a cell, one or more of the virus surface proteins (SU, gp120, Fig. 3) tightly binds to CD4 receptors on the cell membrane.\(^{11,12}\) Next, the trans-membrane protein (TM, gp41, Fig. 3) penetrates the cell membrane and initiates a process of membrane fusion, which allows the core of the virus to enter the cell. Once the genetic material of the virus is in the cytoplasm of the cell, the process of producing DNA complementary to the viral RNA can begin.

1.6.2 Producing and Inserting the Viral DNA (Fig. 6, \(\odot\)).

The reverse transcriptase (RT, p66, Fig. 3) enzyme then polymerises deoxynucleotides on a RNA template, producing single stranded DNA.
After synthesis of a complementary DNA strand, the double-stranded DNA is transported to the nucleus where it helps to insert the double stranded viral DNA into the host cell DNA with the help of HIV integrase (IN, p32, Fig. 3). Once it has been inserted into the host cell gene, this integrated retroviral DNA piece is called a Provirus.

### 1.6.3 From Provirus to mRNA (Fig. 6, 3).

The infected cell may withhold the provirus for many generations without expressing it. However, on appropriate activation RNA molecules corresponding to the total length of the viral genome (Fig. 4) are produced using a cellular polymerase. Some of these molecules of RNA will serve as the genetic material for a new generation of viruses, whilst others acting as mRNA (messenger RNA - carries the genetic information as a sequence of bases from the nuclear DNA to cytoplasm), will serve as templates for protein synthesis. The mRNAs migrate from the nucleus to the ribosomes in the cytoplasm of the cell where the synthesis of large precursor proteins (polyproteins) takes place.

### 1.6.4 The Role of Protease in the Maturation of the Virus (Fig. 6, 8).

All of the proteins, including protease and reverse transcriptase, that will eventually form part of the virus are first produced as a polyprotein. The protease catalyses its own release by hydrolysis of the large polyprotein, which is anchored to the envelope in the immature viral particle.

Once set free, the protease catalyses a series of further hydrolytic cleavages (found in gag and pol sites of polyprotein, Fig. 4), resulting in the final complement of proteins and enzymes for viral maturation\(^{13}\) (Fig. 7).
One long chain of HIV proteins inside infected cell.

Protease

Protease Inhibitor

Two shorter chains of HIV protein

Individual proteins that make new HIV particles

Individual enzymes that help build new HIV particles

**Fig. 7 - Protease Function and Inhibition**

This catalysis is then the main function of the protease. Without HIV-1 protease inhibitors, short chains of HIV proteins and enzymes make new viruses that can infect other cells.

1.6.5 HIV-1 Protease Inhibition (Fig. 6, © & ®).

Protease inhibitors are generally drugs that resemble pieces of the protein chain that the protease normally cuts. By 'gumming up' the protease 'scissors', HIV protease inhibitors can stop the cutting of long chain HIV polyproteins and enzymes into short chains. Protease inhibition results in the formation of 'defective' viruses that cannot go on to infect new cells. Protease inhibitors can greatly reduce the number of new infectious copies of HIV made inside cells, therefore, slowing down the spread of HIV in the body and subsequently the onset of AIDS.
1.7 Structural Properties of HIV-1 Protease.

The retroviral proteases belong to the family of the aspartate proteases, so named because a pair of highly conserved aspartyl residues constitute the catalytic groups at their active sites.

Early predictions regarding the existence, amino acid sequence, three-dimensional structure and role in viral replication of a HIV-1 protease culminated in structural confirmation by X-ray crystallography.

![Fig. 8 - X-ray of HIV-1 Protease (only main backbone).](image)

(Yellow - Trp, Green - Phenylalanine, Blue - Proline, purple - all others.)

Fundamentally, the HIV-1 protease is a 99 amino acid polypeptide monomer that contains an **Asp-Thr-Gly** sequence, usually indicative of an aspartic acid protease (Fig. 9). Unlike a normal aspartic acid protease, which is a monomeric polypeptide containing two such sequences to form a catalytic unit (eg renin), the HIV-1 protease exists as a dimer with each monomer contributing one aspartic acid to the catalytically active site (Fig. 10).
Pro-Gln-Ile-Thr-Leu-Trp-Gln-Arg-Pro-Leu-Val-Thr-Ile-Lys-Ile
Thr-Val-Leu-Glu-Glu-Met-Ser-Leu-Pro-Gly-Arg-Trp-Lys-Pro-Lys
Met-Ile-Gly-Ile-Gly-Ile-Gly-Phe-Ile-Lys-Val-Arg-Gln-Tyr-Asp
Gln-Ile-Leu-Ile-Glu-Ile-Cys-Gly-His-Lys-Ala-Ile-Gly-Thr-Val
Leu-Val-Gly-Pro-Thr-Pro-Val-Asn-Ile-Ile-Gly-Arg-Asn-Leu-Leu
Thr-Gln-Ile-Gly-Cys-Thr-Leu-Asn-Phe

**Fig. 9** - The Amino Acid Sequence of HIV-1 Protease.¹⁷

As required by the accepted catalytic mechanism of aspartyl protease, the two acid residues are in close proximity about the $C_2$ axis of the diad.¹⁶ Consequently, the hydrolyzable bond of peptide substrates must nearly intersect with this symmetry element.

These axis and spatial relationships, coupled with basic transition state theory, have served as a foundation for the design of one class of potent inhibitors of HIV-1 protease.
1.8 Design of Protease Inhibitors.

In the past ten years there have been two main sets of HIV-1 protease inhibitors:

(i) **Peptide derived Inhibitors** - based upon the characterisation of the substrate specificities of the protease and then subsequent synthesis of peptides with similar features. For instance, to form a rational screening process, the hydolyzable amide bond was replaced by nonreactive 'isoteres'. The peptides can be subsequently optimised by modifications in the side chains or backbone. This method can be taken further by adding specific moieties that are capable of forming transition state analogue complexes with the enzyme. Despite the high level of activity in peptide-based drug research, the main obstacle incurred is that of imparting good bioavailability whilst still maintaining pharmacological effect. ¹⁹

(ii) **Non-peptide inhibitors** - based upon discovery through rapid screening of natural products / libraries of synthetic compounds or from structural based design upon crystal structures of HIV-protease / inhibitor complexes. Three dimensional searching of large databases has the ability to discover new leads in drug development programs ²⁰ which when coupled with X-ray crystallographic data encourages de novo design of new inhibitors. Efforts to design inhibitors based on protein structure rather than the protein substrate also offers a further avenue to possible drug candidates.
1.8.1 Peptide Derived Inhibitors.

The HIV-1 protease cleaves peptides found in gag and pol (Fig. 4) polyprotein sites to supply essential proteins and enzymes for viral maturation. Initial studies carried out with peptide sequences from gag and pol sites have identified numerous substrates of the HIV-1 protease.\textsuperscript{21}

In general, the cleavage sites involve the generation of a high energy, tetrahedral diol (1) from a low energy, trigonal amide (Scheme 1).

![Scheme 1 - Generation of Tetrahedral Diol.](image)

The cleavage sites involve a phenylalanine or tyrosine as the N-terminal side (P1) and a proline as the C-terminal (P1') side of the hydolyzable bond (Scheme 1).

Although aspartic proteases may be inactivated by high concentrations of simple epoxides, the absence of an acyl enzyme intermediate in normal catalysis would be expected to severely constrain approaches based on chemical alkylation. So far, attempts to design irreversible inactivators of the HIV protease have yielded only to weak to moderate inhibitors.\textsuperscript{22}

Far greater success has been achieved with competitive inhibitors designed to mimic the transition states leading to (1, Scheme 1), or, more accurately, to act as collected substrate analogues.

The basic principles of this approach were established previously with other aspartic proteases (eg renin, an important target in antihypertensive therapy).\textsuperscript{23}
The results of these earlier studies have been applied to the HIV-1 protease, both directly, by the screening of known inhibitors of renin, eg pepsin, and indirectly, by the incorporation of known transition state analogues into the hydolyzable site of retroviral substrates first reported by Metcalf et al.\textsuperscript{24}

Reduced amide transition state analogues such as (2-5, Table 1) possessed only modest potency, as previously observed with renin, due to a lack of inhibitor / aspartic acid residue interaction.\textsuperscript{25}

Although significant potency has been established for acetyl-pepstatin (6, Table 1), introduction of statine or the statine-analog AHPPA (4-(S)-amino-3-(S)-hydroxy-5-phenylpentanoic acid) into HIV-1 protease cleavage sequences only provided modest inhibitors (7-9, Table 1). Incorporation of the hydroxyethylene transition state isoter has proven to be more effective, particularly the Phe-Gly hydroxyethylene isoter to afford the nanomolar inhibitors (10,11, Table 1).

Insertion of the Leu-Val hydroxyethylene isoter into the gag (Fig. 4) sequence provided inhibitor (12, Table 1) with subnanomolar potency.

Renin inhibitors (13) and (14, Table 1) have served as lead structures for the design of potent hydroxyethylene and dihydroxyethylene-based HIV-1 protease inhibitors, respectively. The hydroxyethylene group probably best mimics the peptide backbone found in substrates and must properly align the important binding elements.

By comparison of inhibitors of pepsin, the use of phosphinate as a tetrahedral transition state mimic led to the potent HIV-1 protease inhibitor (15, Table 1).
<table>
<thead>
<tr>
<th>No.</th>
<th>Structure</th>
<th>Isotere</th>
<th>Kᵢ or IC₅₀ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2)</td>
<td>Ac-Ser-Gln-Asp-Phe(\text{Pro}-\text{Val}-\text{Val}-\text{NH}_2)</td>
<td>Reduced Amide</td>
<td>14,000</td>
</tr>
<tr>
<td>(3)</td>
<td>H-Ser-Ala-Ala-Phe(\text{Pro}-\text{Val}-\text{Val}-\text{NH}_2)</td>
<td>Reduced Amide</td>
<td>1,900</td>
</tr>
<tr>
<td>(4)</td>
<td>H-Val-Ser-Gln-Asp-Phe(\text{Pro}-8n-\text{Val}-\text{OH})</td>
<td>Reduced Amide</td>
<td>3,520</td>
</tr>
<tr>
<td>(5)</td>
<td>Ac-Thr-Leu-Asn-(\text{Na}-\text{Gln}-\text{Arg}-\text{NH}_2)</td>
<td>Reduced Amide</td>
<td>789</td>
</tr>
<tr>
<td>(6)</td>
<td>Ac-Ala-Val(\text{Ala}-\text{Sta}-\text{OH})</td>
<td>Statine</td>
<td>35</td>
</tr>
<tr>
<td>(7)</td>
<td>H-Val-Ser-Gln-Asp(\text{Pro}-\text{Val}-\text{OH})</td>
<td>Statine</td>
<td>3,690</td>
</tr>
<tr>
<td>(8)</td>
<td>Ac-Ser-Gln-Asp(\text{Val}-\text{Val}-\text{NH}_2)</td>
<td>AHPPPA Statine-like</td>
<td>39,000</td>
</tr>
<tr>
<td>(9)</td>
<td>H-Ser-Ala-Ala(\text{Val}-\text{Val}-\text{OH})</td>
<td>AHPPPA Statine-like</td>
<td>810</td>
</tr>
<tr>
<td>(10)</td>
<td>Cbz-Ala-Ala-Phe(\text{Gly}-\text{Val}-\text{Val}-\text{OMe})</td>
<td>Hydroxyethylene</td>
<td>48</td>
</tr>
<tr>
<td>(11)</td>
<td>H-Ala-Ala-Phe(\text{Gly}-\text{Val}-\text{Val}-\text{OMe})</td>
<td>Hydroxyethylene</td>
<td>18</td>
</tr>
<tr>
<td>(12)</td>
<td>H-Val-Ser-Gln-Asp-Leu(\text{Gly}-\text{Val}-\text{Leu}-\text{Val}-\text{OH})</td>
<td>Hydroxyethylene</td>
<td>&lt;1</td>
</tr>
<tr>
<td>(13)</td>
<td>Boc-Phe-Phe-Phe(\text{Phe}-\text{Leu}-\text{Phe}-\text{NH}_2)</td>
<td>Hydroxyethylene</td>
<td>1.0</td>
</tr>
<tr>
<td>(14)</td>
<td>Boc-Phe-His-Leu(\text{Val}-\text{le}-\text{Amp})</td>
<td>Dihydroxyethylene</td>
<td>28</td>
</tr>
<tr>
<td>(15)</td>
<td>Boc-Val-Val-Phe(\text{Pho}-\text{Val}-\text{Val}-\text{NH}_2)</td>
<td>Phosphinate</td>
<td>0.4</td>
</tr>
<tr>
<td>(16)</td>
<td>Ac-Ser-Leu-Asp-Phe(\text{Pro}-\text{le}-\text{Val}-\text{OMe})</td>
<td>Hydroxyethylamine</td>
<td>0.24</td>
</tr>
<tr>
<td>(17)</td>
<td>Cbz-Asn-Phe(\text{Pro}-\text{O}-\text{Bu})</td>
<td>Hydroxyethylamine</td>
<td>140</td>
</tr>
</tbody>
</table>

Amp = 2-(aminomethyl)pyridine

**Table 1 - Peptide-based Inhibitors of HIV-1 Protease**
Hydroxyethylene and phosphinate isosteres incorporating Phe-Pro residues have a greatly reduced potency when compared with their corresponding Phe-Gly analogs. However, incorporation of the Phe-Pro hydroxyethylamine moiety into the p17 / p24 cleavage sequence provided potent inhibitors (16, 17, Table 1).

Systematic structure-activity studies on a number of the above lead compounds have provided inhibitors of unique structure and potency. A summary of these results are now described.

A series of 2-hetero-substituted statine analogues (18, Fig. 11) related to (6, Table 1) have shown inhibitory and antiviral activity ($K_i = 113 \text{ nM}$, $ED_{50} = 0.14 \mu \text{M}$). Phenylnorstatine containing analogues (19a, $R = \text{CH}_3$; 19b, $R = \text{H}$, Fig. 11) showed IC$_{50}$ values of 2.3 and 6.5 nM and $K_i$ values of 2.3 and 5.5 pM, respectively. In infected ATH8 cells, they showed antiviral activity ($ED_{50} \leq 0.1 \mu \text{M}$).

![Fig. 11](image1)

Truncation of the amino terminus of (13) and variations at the P1' - P3' sites (Scheme 1) led to the potent inhibitor (20, $IC_{50} = 0.03 \text{ nM}$, Fig. 12) as well
as the further truncated structure (21, IC$_{50}$ = 0.1 nm, Fig. 12).$^{42}$ Similarly, modification of (12) afforded (22, $K_i$ = 70 nM, Fig. 13)$^{43}$ and optimization of (14, Table 1) led to the subnanomolar inhibitor (23, Fig. 13).$^{44}$

![Chemical Structure](image)

**Fig. 13**

As exemplified by (24, IC$_{50}$ = 0.45 nM, Fig. 14) with the aminohydroxyindane C-terminus,$^{45}$ the para-position of the phenylmethyl sidechain at P1 and P1' sites can be substituted with water solubilizing groups extending out into the enzyme active site.$^{46}$ Tetrahydrofuran and tetrahydropyran urethanes, such as (25, Fig. 14) are also effective inhibitors.$^{47}$

![Chemical Structure](image)

**Fig. 14**

A systematic study of over 100 analogs of (17) provided the potent hydroxyethylamine based inhibitor (26, IC$_{50} < 0.4$ nM, Fig. 15) which proved to be the first HIV-1 protease inhibitor to exhibit clinical efficacy,$^{48}$ which consequently promoted a variety of structural modifications.
Replacement of asparagine with 3'(R)-tetrahydrofuranylglycine gave (27, Fig. 16, Table 2) with improved inhibitory activity (IC\textsubscript{50} = 0.054 nM) and antiviral activity (CIC\textsubscript{95} = 8 nM) in preventing the spread of HIV-1 in MT4 cells.\textsuperscript{49} In an attempt to improve solubility the N-terminus was changed to tetrahydrofuran and tetrahydropyran urethanes such as (28, IC\textsubscript{50} = 160 nM, CIC\textsubscript{95} = 800 nM, Fig. 16, Table 2) but gave a reduction in potency.\textsuperscript{47} The corresponding cyclic sulfone (29, IC\textsubscript{50} = 3 nM, CIC\textsubscript{95} = 50 nM, Fig. 16, Table 2), however, proved to be more effective.\textsuperscript{50} The N-terminal quinoline was replaced by a number of heterocycles, of which (30, IC\textsubscript{50} = 0.07 nM, CIC\textsubscript{95} = 12 nM, Fig. 16, Table 2) showed good activity.\textsuperscript{51} The phenylalanine-derived (31, IC\textsubscript{50} = 5.4 nM, CIC\textsubscript{95} = 200 nM, Fig. 16, Table 2) proved to be an effective inhibitor,\textsuperscript{52} while the hydroxyethylurea derivative (32, IC\textsubscript{50} = 6.3 nM, ED\textsubscript{50} = 10 nM, Fig. 16, Table 2) proved to be less successful.\textsuperscript{53} The trifluoromethylproline-containing (33, IC\textsubscript{50} = 0.05 nM, ED\textsubscript{50} = 0.87 \mu M, Fig. 16, Table 2) showed antiviral activity in HIV-1 infected PBL.
<table>
<thead>
<tr>
<th>No.</th>
<th>R</th>
<th>IC_{50}(nM)</th>
<th>CIC_{95}(nM)</th>
<th>ED_{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>(26)</td>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>-</td>
<td>-</td>
<td>&lt;0.1\mu M</td>
</tr>
<tr>
<td>(27)</td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>0.054</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>(28)</td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td>160</td>
<td>800</td>
<td>-</td>
</tr>
<tr>
<td>(29)</td>
<td><img src="image4" alt="Chemical Structure" /></td>
<td>3</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>(30)</td>
<td><img src="image5" alt="Chemical Structure" /></td>
<td>0.07</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>(31)</td>
<td><img src="image6" alt="Chemical Structure" /></td>
<td>5.4</td>
<td>200</td>
<td>-</td>
</tr>
<tr>
<td>(32)</td>
<td><img src="image7" alt="Chemical Structure" /></td>
<td>6.3</td>
<td>-</td>
<td>10nM</td>
</tr>
<tr>
<td>(33)</td>
<td><img src="image8" alt="Chemical Structure" /></td>
<td>0.05</td>
<td>-</td>
<td>0.87\mu M</td>
</tr>
</tbody>
</table>

**Table 2 - Activity of Analogues of Sequinavir (26)**
Notably less peptide-like in their structure, these Sequinavir (26) based inhibitors are specific for HIV protease and effectively block the proteolytic processing of gag (Fig. 4) proteins in cell culture assays and in clinical trials. The subsite specificity for inhibitors of HIV protease closely resembles that for substrates. Thus, lipophilic, unbranched side chains (especially benzyl and cyclohexylmethyl) are favoured in the symmetry related P1 and P1' subsites while there is a clear preference for Ile, Val or Asn (or the truncated equivalent) at the P2 and P2' side chains (Scheme 1). Planar, aromatic end pieces, which may mimic the peptide backbone rather than the P3 and P3' side chains are an additional common feature of many inhibitors.

A number of structure-based HIV protease inhibitors have been designed and synthesised to complement the C2 symmetric active site of the enzyme. Conceptually, an axis of rotation through the tetrahedral intermediate (1, Scheme 1) was defined, the P' region deleted and the remainder was subjected to a C2 operation to generate symmetric core units. Placement of the axis of symmetry either through the carbonyl carbon or through the middle of the scissile bond provided the lead compounds (34, A74704, IC50 = 3 nM, Fig. 17) and (35, Fig. 17).55

![Fig. 17](image_url)

A related C2 symmetric inhibitor (36, K_i = 0.8 μM, Fig. 18), with isobutyl sidechains, was found to be much weaker.56 Modifications of (35) to evaluate the effect of polar heterocyclic end groups led to non-C2 symmetric A-77003 (37, IC50 ≤ 1 nm, ED50 = 0.2 μM in HIV infected MT4 cells, Fig. 18).

23
In an effort to improve the oral bioavailability, A-80987 (38, $K_i = 0.25$ nM, $ED_{50} = 0.13$ μM in MT4 cells, Fig. 18) a non-symmetric inhibitor, was synthesised and had potent activity and good oral bioavailability. Further improvement was found in the non-symmetric A-84538 (39, $K_i = 15$ pM, $ED_{50} = 30$ nM, Fig. 18). Compound (40, $IC_{50} = 5$ nM, Fig. 18), an oxygen analogue of (34) showed good inhibitory activity, whereas the C2 symmetric phosphinate (41, $K_i = 2.8$ nM, Fig. 18) was found to be a good inhibitor although with only weak antiviral activity.

Fig. 18

High throughput screening identified the penicillin-derived C2 symmetric dimers as good lead structures for analogue optimisation. The dimeric structures (42, $R = CH_2Ph$, $IC_{50} = 0.9$ nM, $ED_{50} = 25$ nM in infected H9 cells, Fig. 19) and (43, $R = Et$, $IC_{50} = 4.8$ nM, $ED_{50} = 5$ μM in MT4 cells, Fig. 19) were both found to be active inhibitors.
A subsequent hybrid of the penicillin derived structure containing a statine residue led to (44, $K_i = 0.25$ nM, $ED_{50} = 4.7$ μM in infected MT4 cells, Fig. 20).59

A great deal of effort has been observed in generating this special class of HIV-1 protease inhibitors over the last ten years through rational design from other proteases. However, no class of drugs is exempt from the problem of oral bioavailability, tissue distribution, metabolism and excretion. These difficulties are particularly acute for peptide-based HIV drugs and as with any peptide-based drug, bridging the gap between in vitro inhibitors and a therapeutic agent will be achieved most quickly by confronting the biological liabilities of peptides from the onset.
1.8.2 Non-Peptide Inhibitors.

Although a number of potent peptidic HIV-1 protease inhibitors have been developed, as described earlier in section 1.8.1, these compounds have liabilities with respect to metabolism and compromised bioavailability.\(^\text{61}\)

Efforts have been made in several laboratories to develop non-peptide based HIV-1 protease inhibitors but to date such compounds have not reached the same level of potency as the best peptidomimetic inhibitors.

There is, as a result, a continuing need for lead compounds for discovery of the development of non-peptide HIV-1 protease inhibitors through three-dimensional searching of large databases\(^\text{62}\) or by other means.

Cytochalasin H (45, \(K_i = 1 \mu M\), Fig. 21) was isolated from Hypoxylon fragifome, a bark-inhabiting Ascomycetes (fungal spore), which was shown to be a competitive inhibitor.\(^\text{63}\) Didemnaketals A (46, \(R = \text{COCH}_3\), \(IC_{50} = 2\mu M\), Fig. 21) and B (46, \(R = \text{C(\text{CH}_3)\text{=CHCH}_2\text{CH}_2\text{CH(\text{CH}_3)}\text{CH}_2\text{CO}_2\text{CH}_3}\), \(IC_{50} = 10 \mu M\), Fig. 21) were isolated from Didemnum sponge.

![Figure 21](image)

A number of brominated polyacetylenic acids were isolated from the marine sponge Xestospongia muta, such as the acid (47, \(IC_{50} = 7 \mu M\), Fig. 22), and showed varying activity.\(^\text{64}\) Various cation complexes of cycloprazonic acid (48, CPA, Fig. 22) were found to be competitive inhibitors, specifically CPA\(_2\)Tb\(^{2+}\) (\(K_i = 6 \text{ pM}\)) showing very high inhibitory activity.
After molecular modelling studies using Quanta™, a novel trans-3-oxabicyclo[3.3.0] system was identified as a prime low molecular weight target in an attempt to improve bioavailability. Various analogues of (49, Fig. 23) and (50, Fig. 23), which were synthesised, displayed weak activity (IC₅₀ = 70-100 μM).

![Fig. 22](image)

From random screening of sample collections by two groups, 4-hydroxybenzopyran-2-one (51, Kᵢ = 0.8 μM, Fig. 24) and 4-hydroxypyran-2-one (52, Kᵢ = 38 nM, Fig. 24) residues were identified as HIV-1 protease active.

![Fig. 23](image)

Subsequent structure activity probing led to the sulfonamide-substituted cyclooctopyranones (53, R = Et, X = H, Kᵢ = 75 nM; R = cyclopropane, X = H,
$K_i = 15 \text{ nM}; R = \text{cyclopropane}, X = \text{NHCO(CH}_2)_2\text{NHCO}_2^\text{Bu}, K_i = 4 \text{ nM, Fig. 25}$ and specifically the $p$-cyanophenyl sulfonamide derivative ($54, K_i = 0.8 \text{ nM, ED}_{50} = 1.3 \mu\text{M, Fig. 25}$).

![Chemical structures](image)

**Fig. 25**

A computational search of the Cambridge Structural Database$^{68}$ based on complementary shapes of molecules with the HIV-1 protease active site led to the antipsychotic agent haloperidol ($55, X = 0, K_i = 100 \mu\text{M, Fig. 26}$). Although only a weak inhibitor of HIV protease, the novel structure has served as a template for the design of other non-peptide structures. The 1,3-dithiolane derivative ($55, X = \text{-SCH}_2\text{CH}_2\text{S-}, K_i = 15 \mu\text{M, Fig. 26}$) was found to be a more active inhibitor.$^{69}$ In an attempt to synthesise irreversible inhibitors of HIV-1 protease, a FMOC based epoxide$^{70}$ ($56, K_{\text{inact}} = 65 \mu\text{M, Fig. 26}$) of haloperidol ($55, X = 0, \text{Fig. 26}$) has been made. This compound showed that the introduction of an epoxide functionality can specifically alkylate the catalytic aspartate residues associated with the active site and, therefore, serve as a template for future work.

![Chemical structures](image)

**Fig. 26**

In another study,$^{71}$ a computer-assisted substructure search was based upon the crystal structure of HIV-1 protease / (34, Fig. 17) complex, and ($57, IC_{50} = 11 \mu\text{M, Fig. 27}$) was found to have inhibitory activity.
Searching compound databases for potential molecules with one oxygen displacing the flap-associated water and a second oxygen interacting with the two catalytic aspartic acid residues led to *trans*-1,4-cyclohexanediols and hydroquinones as targets. Compounds (58, \( R = OH, X = C(O), K_i = 10 \mu M; R = CH_2OH, X = S(O), K_i = 7 \mu M, \) Fig. 27) were subsequently synthesised and showed moderate HIV-1 protease activity.\(^{72}\)

![Fig. 27](image)

Lam and co-workers\(^{73}\) reported a novel approach to HIV-1 protease inhibition based upon a 7-membered cyclic urea scaffold. These compounds have nanomolar binding affinities and are orally bioavailable. The fundamental characteristic of these types of inhibitors eg DMP323 (59, \( K_i = 0.33 \text{nM}, \) Fig. 28) and the aza-backbone modified A-98881 (60, \( K_i = 0.05 \text{nM}, EC_{50} = 0.002 \mu M, \) Fig. 28),\(^{74}\) is the cyclic urea carbonyl mimicking the structural water molecule involved in the HIV-1 protease site.\(^{75}\)

![Fig. 28](image)
The crystal structure of DMP 323 (59, Fig. 28) bound in the active site of HIV-1 protease was solved at 1.8 Å resolution. The inhibitor is bound symmetrically (Fig. 29) in the active site with the urea oxygen accepting two hydrogen bonds from the flap residues Ile 50 / 50'.

The p-hydroxymethyl substituent at P2 picks up an additional hydrogen bond from the backbone NH of Asp 29 / 29' and Asp 30 / 30'. The diol oxygens interact with the catalytic aspartate region.

The cyclic urea provides a rigid scaffold for accurate alignment of all the key elements with their corresponding binding motifs.

Subsequent structural analysis probing\textsuperscript{76,77,78} has given rise to more potent analogues (Fig. 30 and Table 3) and a better insight into the structural properties of the HIV-1 site.
<table>
<thead>
<tr>
<th>No.</th>
<th>( R_1 )</th>
<th>( R_2 )</th>
<th>( K_i (\text{nm}) )</th>
<th>( IC_{50} (\text{nM}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>(61)</td>
<td>( m-\text{CH}_3\text{C(O)C}_6\text{H}_4 )</td>
<td>( m-\text{CH}_3\text{C(O)C}_6\text{H}_4 )</td>
<td>0.11</td>
<td>37</td>
</tr>
<tr>
<td>(62)</td>
<td>( m-\text{CH}_3\text{C(O)C}_6\text{H}_4 )</td>
<td>cyclopropylmethyl</td>
<td>0.87</td>
<td>650</td>
</tr>
<tr>
<td>(63)</td>
<td>3,4-(ethylenedioxy)phenyl</td>
<td>( m-\text{CH}_3\text{C(O)C}_6\text{H}_4 )</td>
<td>0.04</td>
<td>4.2</td>
</tr>
<tr>
<td>(64)</td>
<td>3,4-(ethylenedioxy)phenyl</td>
<td>( m-\text{CH}_3\text{NH}C_6\text{H}_4 )</td>
<td>0.14</td>
<td>8.7</td>
</tr>
<tr>
<td>(65)</td>
<td>2,3-dihydro-5-benzofuranyl</td>
<td>( m-\text{CH}_3\text{C(O)C}_6\text{H}_4 )</td>
<td>0.048</td>
<td>10</td>
</tr>
<tr>
<td>(66)</td>
<td>3,4-(methylenedioxy)phenyl</td>
<td>( m-\text{CH}_3\text{C(O)C}_6\text{H}_4 )</td>
<td>0.043</td>
<td>14.7</td>
</tr>
<tr>
<td>(67)</td>
<td>4-methyl-3,4-dihydro-6-benzoaxazinyl</td>
<td>( m-\text{CH}_3\text{C(O)C}_6\text{H}_4 )</td>
<td>0.08</td>
<td>16.4</td>
</tr>
<tr>
<td>(68)</td>
<td>benzyl</td>
<td></td>
<td>0.027</td>
<td>4.2</td>
</tr>
<tr>
<td>(69)</td>
<td>benzyl</td>
<td></td>
<td>0.024</td>
<td>5.1</td>
</tr>
</tbody>
</table>

**Table 3** - Structural Activity Probing of 7-Membered Cyclic Urea
1.8.3 Combination of Protease and Reverse Transcriptase Inhibitors.

Five reverse transcriptase inhibitors, zidovudine (70, AZT, Fig. 31), didanosine (71, ddl, Fig. 31), stavudine (72, d4T, Fig. 31), zalcitabine, (73, ddC, Fig. 31) and lamivudine (74, 3TC, Fig. 31) have been approved for treatment of HIV infection.

![Chemical structures of reverse transcriptase inhibitors](image)

**Fig. 31**

In AIDS patients, these drugs initially reduce viral load. However, adverse side effects are associated with extensive use of nucleoside analogues. In addition, the appearance of virus with reduced sensitivity renders the drugs less effective over time. A second or even a third inhibitor can be used in combination and is now the standard therapy.

The rationale of combination therapy for treatment of HIV infection includes increased efficacy, reduced toxicity, dose reduction and delay of emergence of resistant strains. A number of reports have described *in vitro* experiments in which HIV protease inhibitors were shown to act synergistically with nucleoside and non-nucleoside reverse transcriptase inhibitors using various viral strains in different cell types. This has now been clinically proven.
Because protease inhibitors and reverse transcriptase inhibitors work on two separate steps in the HIV replication process, one of the better ways to use a protease inhibitor is to combine it with at least one or, if possible, two reverse transcriptase inhibitors resulting in a synergistic effect.

Synergy can result from combining drugs that affect more than one cell type or cells in different stages of activation, or inhibit virus replication at different steps.\textsuperscript{82} Synergy may also be observed when combining antiviral agents with compounds that enhance drug uptake, affect cell activation levels, or increase the level of an active drug metabolite.

Compound (26, Fig. 15) was found to be synergistic with AZT or ddC in CEM-T4 cells infected with HIV-1 strain GB8.\textsuperscript{83} Subsequent studies and development led to a treatment using a cocktail of drugs (AZT, ddC and 26, Fig. 15). This is currently one of the leading treatments for HIV patients. When patients took Indinavir (21, Fig. 12) plus AZT (70, Fig. 31) and 3TC (74, Fig. 31), the levels of virus in their blood dropped by 100-fold over a period of six months. Not only did the viral loads drop but patients who took this particular cocktail also had substantial, and sustained, increases in CD4+ T cell counts.\textsuperscript{84}

Many drug combinations are at clinical trial stage at the moment, emphasising the need for both new reverse transcriptase and protease inhibitors with differing properties. One set of compounds showing different HIV-1 transcriptase and protease inhibitory activity is the steroids.
1.9 Steroids.

Fig. 32 - Steroidal nucleus.

The steroid system, selected by the evolutionary process to perform some of the most fundamental biological functions, has become one of the most intriguing classes of biologically active compounds. They include a wide range of naturally occurring compounds among which are the sterols, the bile acids, the sex hormones, vitamin D₃, the adrenocortical hormones, the cardiac glycosides, the sapogenins, some alkaloids and other minor groups. Fig. 32 shows the backbone of the 5α- and 5α-cholestanes.

Steroidal prodrugs, (75, Fig. 33) and (76, Fig. 33), of AZT (70, Fig. 31) have been synthesised and tested, employing the special properties of steroids (eg increased plasma half life, lipophilicity, cell uptake, decreased toxicity and enhanced cell permeability), to try and increase efficacy.
The therapeutic indices of all of the prodrugs illustrated in Fig. 33 were comparable to that of AZT. Steroidal inhibitors of the HIV-1 nuclear regulatory protein tat (Fig. 4) based upon keto / enol epoxides, (77, Fig. 34) and (78, Fig. 34) have been located from a compound library screening. The steroid nucleus is becoming a versatile scaffold for the design and synthesis of new classes of inhibitors in HIV and other viruses.
1.10 Design of Steroidal Inhibitors of HIV-1 Protease.

Peptidomimetics have emerged as an active field at the interface of bioorganic, organic and medicinal chemistry. The interest derives from the expectation that such molecules will have both better biostability and oral bioavailability than their peptide counterparts.

Several groups have reported on the design and synthesis of novel scaffolds. However, these early compounds were not designed for specific receptors, as they lacked relevant peptidal side chains that would permit biological testing, their conformations probed solely by physical measurement. Recently, Hirschmann et al. reported the first design and synthesis of a peptidomimetic employing a steroidal scaffold in an attempt to bind to the fibrinogen receptor on blood platelets (GP IIb / IIIa). Antagonists were designed on an androstane backbone to fit the same approximate area of a β-turn (79, Fig. 35) believed to be involved in the residues Arg-Gly-Asp contained in known inhibitors. This resulted in compound (80, IC\(_{50}\) = 100μM, Fig. 35) showing activity in a GP IIb / IIa-fibrinogen binding assay.

Eddolls and Marples designed and synthesised some potential analgesics (81, Fig. 36) and (82, Fig. 36) based upon amino estratrienes as peptidomimetics.
In the past, many steroids have been used as model substrates for a myriad of reactions due to their stability and robustness.

We have used the conformation of inhibitor A74704 (34, Fig. 17) in its HIV-1 protease complex$^5$ to design steroidal HIV-1 protease inhibitor alternatives which have a number of features in common. The targets are 11-amino-12-hydroxy-steroids based upon estra-1,3,5(10)-triene incorporating a phenyl residue or a benzyl residue at C-17 or C-18. In these structures, the aromatic A ring and the phenyl residue in the C/D region mimic the desirable benzyl group side chains and the 11-amino-12-hydroxy moiety mimics the hydroxyethylamine present in A74704 (34, Fig. 17). Figs. 38 - 40 show best fit superimpositions of potential steroid targets (83, Fig. 37) and (84, Fig. 37) with A74704 (34, Fig. 17) using energy minimisations. This modelling was performed with SYBIL ver. 5.5 molecular modelling package$^6$ (protons not shown, red - Oxygen atoms, blue - Nitrogen atoms, white - Carbon atoms).
Fig. 38 - Superimposition of steroid (83, white / blue / red) on A74704 (34, green).

Fig. 39 - Superimposition of steroid (84, n = 0, white / blue / red) on A74704 (34, green).

Fig. 40 - Superimposition of steroid (84, n = 1, white / blue / red) on A74704 (34, green).
It is of particular interest to note the close superimposition of the aromatic rings, the hydrophobic groups attached to the nitrogen and the important carbonyl groups which hydrogen bond to a water molecule in the active site in both A74704 (34) and the sterodal inhibitors (83, 84). The anticipated increased lipophilicity of the target steroids may offer significantly improved *in vivo* activity as has been observed in some renin inhibitors.\(^9\)

Some of the key features surveyed with respect to the use of a steroid nucleus as a scaffold for HIV-1 protease inhibitor design are:-

- the volume matches that of the backbone of A74704 (34, Fig. 17);
- many steroids are already drugs with excellent bioavailability;
- the rigid steroid nucleus should reduce the tendency for hydrophobic collapse;
- a large body of literature permits regio- and stereoselective introduction of functionality;
- the rigid conformation of the steroid nucleus would allow structure activity relationships and changes in acceptable geometry of molecules in the active site to be probed meaningfully.

A related approach was reported earlier (Section 1.8.2), towards the identification of non-peptide HIV-1 protease inhibitors,\(^7\) which led to the investigation of haloperidol (55, X = O, Fig. 26). Fig. 41 shows an attempt to superimpose a suitable conformer of haloperidol (55, X = O, Fig. 26) on A74704 (34, Fig. 17).
Fig. 41 - Superimposition of (55, white / blue / red) on A74704 (34, green).

Clearly the fit is poor, but the fact that some activity has been reported for (55, X = O, Fig. 26) suggests that some flexibility is possible within the HIV-1 protease active site. The proposed targets have been shown to fit into the active site of the HIV-1 protease and Fig. 42 shows this for (84, n=1).

Fig. 42 - Steroid (84, n=1) fit into HIV-1 protease site.
Removing the functionality from C-16 and C-18 of (83, Fig. 37) and (84, Fig. 37) results in the primary target (85, Fig. 43).

![Chemical structures](image)

**Fig. 43**

Saturation in the A-ring as in (86, Fig. 43) gives a 'cyclohexylmethyl' component found in some inhibitors, for example (22, Fig. 13) and (23, Fig. 13). Both (85, Fig. 43) and (86, Fig. 43) are acceptable targets from readily available starting materials. It is hoped that the rigid steroidal nucleus coupled with the C-ring functionality might prove sufficient to mimic the HIV-1 protease inhibitor (34, Fig. 17).

An obvious starting point would be to take an estrogen (eg estradiol, 87, Scheme 2) with the A-ring already aromatised and to try and introduce the 11-amino-12-hydroxy moiety (Scheme 2) and synthesise compounds of the type (85, Fig. 43). Ongoing work within the group, showed some problems in preparing materials early on in the synthesis, including the insertion of nitrogen functionality into position C-11 and subsequent oxygenation at C-12. Generation of the key $\Delta^{9,11}$-alkene intermediate (89, Scheme 2) enabled exploration of both electrophilic and nucleophilic nitrogen addition reactions but results were disappointing. Scheme 2 shows possible approaches to compounds (85).
Scheme 2 - (i) Dimethyldioxirane, Me₂CO, 0 °C, 75 %; (ii) Oxalic acid, PhH 80 °C, 97 % \( \Delta^{8(11)}: \Delta^{8,9} = 95:5 \); (iii) mCPBA, Na₂CO₃, CHCl₃, 59 % α: β = 7:1; (iv) NaN₃, EtOH, H₂O, NH₄Cl, 12 %; (v) H⁺; (vi) SeO₂; (vii) NaBH₄; (v) TBDMSCI, imidazole; (ix) H₂, catalyst; (x) ceric ammonium nitrate, HOAc H₂O, LiCl; (xi) OH⁻; (xii) Boc₂O, DMAP.
An alternative approach from the readily available bile acid, deoxycholic acid (97, Fig. 44), in which the 12-oxygen is already present, looked promising and versatile, and should allow the necessary incorporation of the 11-nitrogen function.

![Chemical structure](image)

Fig. 44

It was envisaged that the side chain could also be modified to give a range of structures with varying degrees of lipophilicity. In the bile acid series, of course, the A-ring aromatic residue is replaced by a cyclohexane moiety and this may be acceptable or even beneficial for activity.

Aromatisation of the A-ring in principle may be achieved by manipulation of suitably substituted C-ring intermediates. One main advantage of pursuing this route is that all the compounds may be tested for biological activity as soon as the 11-amino-12-oxo/hydroxy functionality has been introduced. Consequently, the active site may then be probed by appropriate structure variation, including unsaturation of the A-ring.
Chapter 2.
2.1 Synthetic Strategy.

This chapter serves only to present a broad outline of the synthetic strategies designed to obtain the target 11-amino-12-hydroxy compounds (85 and 86, Fig. 43).

\[ \text{(85)} \quad \text{(86)} \]

2.2 Routes to Target Steroid (86) through Intermediate (100), (Schemes 3 and 4).

Compound (100, Scheme 3) was chosen as a key intermediate for one route to 11-amino-protected-12-hydroxy bile ester (86). To obtain this intermediate (100), a number of steps were proposed starting from commercially available deoxycholic acid (97). The ketone (100) could, in principle, be prepared by oxidation of (99). Derived from selective protection of the dihydroxy compound (98), intermediate (100) would provide the foundation of much of the synthesis to follow.

Having obtained (100), introduction of a nitrogen functionality at C-11 might best be achieved using nitrosation (step i, Scheme 4), to give the 11-oximino compound (101). The 11-oximino-12-ketone (101) could then be reduced using complex metal hydrides (step x, Scheme 4). Catalytic hydrogenation and subsequent protection of the 11-amine (108) would lead to the target bile ester (86) (steps vii and ix, Scheme 4).
Scheme 3 - Routes to intermediates (100) and (110).

Possible synthetic steps -

(i) ROH, H⁺ or RNH₂, activating agent; (ii) (R₁)₂CO or R¹, DMAP, py; (iii) Na₂Cr₂O₇;
(iv) MsCl, py; (v) KOAc, py.
Direct nitrogen functionality may also be introduced into C-11 of (100) via an electrophilic amination \(^{102}\) (step iii, Scheme 4) utilising DEAD (diethyl azodicarboxylate) or DBAD (di-tert-butyl azodicarboxylate). The hydrazide (103), a possible anti-viral agent in its own right could be hydrogenated selectively to the protected amino ketone (109) by treatment with Raney Ni / H\(_2\) in methanol \(^{103}\) (step vii, Scheme 4). Subsequent metal hydride reduction of 12-ketone (109) (step x, Scheme 4) should lead to the desired bile ester target (86).

Another alternative route (via step ii, Scheme 4) involved the secondary intermediate \(\alpha\)-bromo ketone (102). We proposed that bromination of 12-ketone (100) under standard conditions \(^{104}\) would lead to compound (102) (step ii, Scheme 4) and subsequent S\(_N\)2 displacement of the bromine with an azide ion \(^{105}\) (step v, Scheme 4) would introduce a nitrogen functionality at C-11. Hydrogenation of azide \(^{106}\) (105) to amine (107) (step vii, Scheme 4), amino mono-protection (step ix, Scheme 4) and 12-keto reduction (step x, Scheme 4) would give bile ester target (86).

A second route from the \(\alpha\)-bromo ketone (102) could involve a base catalysed ketol rearrangement \(^{107}\) (step iv, Scheme 4) to give 12-hydroxy-11-keto steroid (104). Subsequent oximation \(^{108}\) (using hydroxylamine hydrochloride or substituted derivatives) of the 12-hydroxy-11-ketone (104) would lead to the 12-hydroxy-11-oxime (106) (step vi, Scheme 4). Oxime reduction to the amino alcohol (108) and amino protection would give target compound (86).
Scheme 4 - Routes to (86) from 12-Keto (100).

Possible synthetic steps -

(i) C₅H₁₁ONO, KO'Bu; (ii) Br₂, HOAc; (iii) DEAD or DBAD, LDA, THF, -78 °C;
(iv) OH⁻; (v) NaN₃ or HN₃, DMSO or EtOH; (vi) NH₂OR₂.HCl, py; (vii) H₂, catalyst;
(viii) Zn, HCl; (ix) Boc₂O, DMAP; (x) NaBH₄, CeCl₃, EtOH.
2.3 Routes to Target Steroid (86) through Intermediate (110), (Schemes 3 and 5).

Alkene (110, Scheme 3) was chosen as a key intermediate for another route to 11-amino-protected-12-hydroxy bile ester (86). Preparation of (110) could be accomplished from (99, Scheme 3) by forming a sulphonate ester at C-12 (step iv, Scheme 3) and subsequent elimination (in basic media at elevated temperature, step v, Scheme 3).

It was envisaged that direct insertion of cis-hydroxyamino function may be facilitated by an osmium catalysed asymmetric aminohydroxylation (step i, Scheme 5) to form target compound (86). However, stereochemistry around C-11 and C-12 would probably be α.

Peracid epoxidation of olefin (110) (step ii, Scheme 5) would provide an alternative key intermediate in the synthesis of (86). We proposed to cleave the epoxide ring with nitrogen nucleophiles (steps iii and viii, Scheme 5) to give the nitrogen functionalised alcohols (112) and (115) respectively. It was anticipated that azide ion (step iii, Scheme 5) might yield the azido alcohol (112) or alternatively, a primary amine (step viii, Scheme 5) could give an amino alcohol (115). Conceivably, inversion of the stereochemistry at the C12-OH could be achieved via the Mitsunobu conditions.

A possible alternative to the ring opening of epoxides with azide / amines, is the addition of acetonitrile in the presence of a Lewis acid to form an oxazoline (113) (step iv, Scheme 5). Subsequent acid catalysed hydrolysis (step vi, Scheme 5) could provide the amino alcohol (114). Protection (step vii, Scheme 5) and manipulation of the stereochemistry (step ix, Scheme 5) could lead to the desired target compound (86).
Scheme 5 - Routes to (86) from Δ¹¹ (110).

Possible synthetic steps -

(i) TsNCINa.3H₂O, OsO₄, 'BuOH; (ii) mCPBA, CHCl₃, Na₂CO₃; (iii) NaN₃ or HN₃, DMSO or EtOH; (iv) CH₃CN, Lewis acid; (v) H₂, catalyst; (vi) H⁺; (vii) Boc₂O, DMAP, py; (viii) R₂NH₂; (ix) Ph₃P, DEAD, PhH.
2.4 Routes to Target Steroid (85) from (86), (Scheme 6).

Once C-ring oxygen and nitrogen functionality have been introduced, aromatisation of the A-ring to (85) was envisaged. Protection of the 12-OH (86) with t-butyldimethylsilyl chloride (TBDMSCl) and imidazole, (step i, Scheme 6), would allow selective deprotection of 3-OH (step ii, Scheme 6) and a chromium mediated oxidation to the 3-keto compound (117). It was envisaged that (117) could be converted to the Δ¹⁴-3-ketone¹¹³ (118) (step iv, Scheme 6). Aromatisation of (118) in ‘activated’ zinc and ethanol,¹¹⁴ with expulsion of the angular methyl group, would lead to estratriene (119) (step v, Scheme 6). Simple protection of the phenol would then give target compound (85).
Scheme 6 - Formation of (85) through unsaturation of (86).

Possible synthetic steps -

(i) TBDMSCl, imidazole; (ii) OH⁺; (iii) Na₂Cr₂O₇; (iv) Br₂, dioxan, LiBr, Li₂CO₃, DMF; (v) Zn, EtOH; (vi) Ac₂O, py.
2.5 Routes to Target Steroid (85) through Intermediate (125) and (126), (Schemes 7 and 8).

An alternative approach to target (85) was envisaged involving aromatisation of the A-ring then utilising the oxygen function at C-12 to introduce a nitrogen function at C-11.

The known Oppenauer oxidation\textsuperscript{115} of methyl deoxycholate (98) would lead to 3-OH being oxidised selectively (step i, Scheme 7). We conceived preparation of $\Delta^{11}$ olefin (122) via elimination of the sulphonate ester (121) at C-12 (step ii and iii, Scheme 7). Conversion to the dienone (123) by standard conditions\textsuperscript{116} (step iv, Scheme 7) and peracid epoxidation could give it's epoxy derivative (124) (step v, Scheme 7). Treatment of both (123) and (124) with 'activated' zinc in ethanol could give their aromatic derivatives (125) and (126) respectively.

Scheme 8 is analogous to Scheme 5 and outlines possible alternative routes to (85).
Scheme 7 - Formation of estrogenic intermediates (125) and (126) from (98).

Possible synthetic steps - (i) Al(O'Pr)$_3$, cyclohexanone; (ii) MsCl, py; (iii) KOAc, py; SeO$_2$ or Br$_2$, dioxan; Li$_2$CO$_3$, DMF; (v) Zn, EtOH.
Scheme 8 - Routes to (85) through epoxide (126) or Δ¹¹ (125).

Possible synthetic steps -

(i) mCPBA, CHCl₃, Na₂CO₃; (ii) Ac₂O, py; (iii) TsNCINa·3H₂O, OsO₄, tBuOH;
(iv) NaN₃ or HN₃, DMSO or EtOH; (v) CH₃CN, Lewis acid; (vi) R²NH₂; (vii) Ph₃P, DEAD, PhH;
(viii) H₂, catalyst; (ix) H⁺; (x) Ph₃P, DEAD, PhH.
2.6 Routes to Target Steroid (85) through Intermediates (135) and (136), (Schemes 9 and 10).

A route to (85) could be envisaged through the aromatic 12-ketone (135, Scheme 9) or the aromatic $\alpha,\beta$-unsaturated ketone (136, Scheme 9), by means of aromatisation of the A-ring then introduction of a nitrogen functionality through the ketone.

Starting with methyl deoxycholate (98), conversion to the known 1,4-dien-3,12-dione (133) and 1,4,9(11)-trien-3,12-dione (134) could be achieved by suitable oxidation procedures (Scheme 9). Treatment of both (133) and (134) with 'activated' zinc in ethanol could give their estrogenic derivatives (135) and (136) respectively (step iii, Scheme 9).

Manipulation of the aromatic 12-ketone (135, Scheme 9) to target (85) could be achieved through steps analogous to that of the saturated 12-ketone (100, Scheme 6) as discussed in section 2.4.

We proposed reduction of $\alpha,\beta$-unsaturated ketone (138) could give the allylic alcohol (139, step iii, Scheme 10) and subsequent transformation to the trichloroimidate (141) (step v, Scheme 10). Intramolecular cyclisation of the imidate (141) could lead to the oxazoline (144, step vi, Scheme 10) which through hydrolysis (step vii, Scheme 10), followed by radical deiodination (step viii, Scheme 10) could lead to the hydroxy-trichloroacetamide (143). De-protection (step ix, Scheme 10) then appropriate protection (step x, Scheme 10) of the 11-amino group in (143) would yield the estrogen target (85).
Scheme 9 - Formation of estrogenic intermediates (135) and (136) from (98).

Possible synthetic steps -

(i) Na$_2$Cr$_2$O$_7$, H$_2$O, H$_2$SO$_4$; (ii) SeO$_2$; (iii) Zn, EtOH.
Scheme 10 - Routes to (85) through 12-keto (135) or α,β-unsat. ketone (136).

Possible synthetic steps -
(i) Ac₂O, py; (ii) H₂ catalyst; (iii) NaBH₄, CeCl₃; (iv) DEAD or DBAD, LDA, THF, -78 °C;
(v) Cl₂CN, NaH; (vi) NIS; (vii) HClO₄, MeOH; (viii) Bu₃SnH, AIBN; (ix) H₂NNH₂, EtOH;
(x) Boc₂O, DMAP.
Chapter 3.
Discussion.
3.1 Routes to Target Steroid (86) through Intermediate (100), (Schemes 3 and 4).

The key intermediate (100) was obtained in three steps from deoxycholic acid (97, Scheme 3, Section 2.2).

Previously, deoxycholic acid (97) had been esterified by either refluxing methanol with an acid catalyst,\textsuperscript{120} or with the use of a microwave oven.\textsuperscript{121} For our purposes, deoxycholic acid (97) was methylated with freshly prepared diazomethane in THF\textsuperscript{122} giving, after chromatography, methyl deoxycholate (98, R = OMe, 99\%) as colourless needles.\textsuperscript{123} The $^1$H NMR spectrum of (98, R = OMe) gave a singlet for the methyl ester $\delta_H$ 3.65 ppm (CO$_2$CH$_3$) and IR spectrum gave an ester carbonyl stretch band at $\nu_{\text{max}}$ 1738 cm$^{-1}$. Esterification with diazomethane is dependent upon the dissociation of a proton from (97), to leave the carboxylate ion possessing a lower free energy. The hydroxyl protons on C3-OH and C12-OH are not as labile as the carboxylic acid due to the relative instability of the alkoxide ion which would be formed and are therefore not alkylated.

Modification of the bile acid side chain was chosen, at this point, as a model study to assess the ease at which the lipophilicity of the potential inhibitors may be changed. The amides of deoxycholic acid (97) were selected for synthesis due to the numerous methods in which they can be made. The classic mixed carbonic anhydride procedure using chloroformate necessarily involves separate activation and aminolysis steps, but mixed ethyl carbonic anhydrides can also be generated by the reaction of carboxylic acids with EEDQ (145, N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline)\textsuperscript{124} and this can be done in the presence of an amino component (Scheme 11). Reagent (145) can therefore be used as a direct coupling reagent because, in this technique, the mixed carbonic anhydride is consumed by aminolysis as soon as it is formed and the opportunity for the intervention of side reactions is therefore minimal.
Since the co-products are quinoline (146, Scheme 11), ethanol and carbon dioxide, workup is simple. Benzylaminodeoxycholic acid (98, R = HN-CH₂-Ph, 93%) was prepared by refluxing benzylamine hydrochloride with EEDQ (145) and deoxycholic acid (97) in ethyl acetate. The ¹H NMR spectrum of (98, R = HN-CH₂-Ph) showed a quartet at δ 4.4 ppm (J = 6 Hz) corresponding to the benzylic methylene, broad NH peak at δ 5.8 ppm and five aromatic protons at δ 7.2 ppm. The ¹³C NMR spectrum gave an amide carbonyl at 173.7 ppm and the corresponding IR spectrum gave an amide carbonyl stretch (-CO-NH-) at νmax 1648 cm⁻¹.

The glycine ethyl ester conjugate (98, R = NH-CH₂-CO₂C₂H₅, 92%) was obtained using the same method with EEDQ (145), the structure of which was supported by the ¹H NMR spectrum; δ₂ 1.20 ppm (triplet, J = 3 Hz, OCH₂CH₃), 3.90 ppm (doublet, J = 5 Hz, HN-CH₂-CO), 4.11 ppm (quartet, J = 7 Hz, OCH₂CH₃) and a singlet at 6.26 ppm for the NH functionality.

The methyl ester (98, R = Me) proved to be the easiest to prepare in bulk and the most versatile (having potential to be modified by trans-amidation / esterification after functionality in the C-ring had been introduced) and therefore, was chosen as the main protecting group at C-24 for the rest of the synthetic pathway. However, benzylaminodeoxycholic acid (98, R = HN-CH₂-Ph) and glycine ethyl ester conjugate (98, R = NH-CH₂-CO₂C₂H₅) were taken through some of the pathway (Scheme 3 and Scheme 4) in parallel with the methyl ester (98, R = OMe).
Attempts at mono-protection of C3α-OH over C12α-OH began with the known acetylation, using acetic anhydride in pyridine at room temperature, provided the desired product (99a, 42%)\textsuperscript{125} which gave characteristic peaks in the $^1$H NMR spectrum at $\delta$ 2.04 ppm (singlet, $\text{CH}_3\text{CO}$) and 4.67 ppm (multiplet, $\text{CH}-3\beta$). However, a significant amount of the diacetylated product (99b, 22%) was present, making purification using flash column chromatography difficult due to the similarity in $R_f$ values.

Increasing the size of protecting group to TBDMS (tert-butyldimethylsilyl) would predictively yield a higher proportion of mono-protected over di-protected hydroxy-steroid as the C-12 position is much more sterically hindered than that of C-3 (Fig. 45).
The steric bulk which accounts for the relative stability of TBDMS ethers also hinders their formation, therefore, catalytic amounts of imidazole are added. The TBDMSCI reacts with imidazole to give the intermediate (146, Fig. 46) below, which in its protonated form is a highly reactive silylating reagent.

When (146) was reacted with (98, R = OMe), the exclusive product isolated was the mono-silylated steroid (99c, 73%) after recrystallisation from ethyl acetate as colourless needles. The NMR spectra of (99c) showed characteristic signals at $\delta_\text{H} 0.05$ (singlet, Si(CH$_3$)$_2$), 0.88 (multiplet, CH$_3$-19 + SiC(CH$_3$)$_3$), 3.53 (multiplet, CH-3$\beta$) ppm in the $^1$H NMR spectrum and $\delta_\text{C} - 4.64$ (Si(CH$_3$)$_2$), 18.27 (SiC(CH$_3$)$_3$) and 25.96 (SiC(CH$_3$)$_3$) ppm in the $^{13}$C NMR spectrum. This was conclusive of the incorporation of TBDMS.

An alternative protection was provided by the use of the benzoyl group (addition of benzoyl chloride to (98, R = OMe) in refluxing pyridine).

This led to a slight improvement in yield of the mono-protected steroid (99d, 84%) with only a small amount of dibenzoylated product being formed (99e, 2%). The $^1$H NMR spectrum of (99d) revealed a downfield shift of CH-3$\beta$ to $\delta_\text{H} 4.8$ ppm and confirmed the presence of a mono-substituted aromatic ring by signals between 7 - 8 ppm. A slight increase in concentration of benzoyl chloride added (1.3 equivalents) led to the formation of a higher proportion
of di-protected product (99e, 31 %, \( ^1 \)H NMR spectrum \( \delta \)H 4.88 (multiplet, CH-3\( \beta \)), 5.38 (singlet, CH-12\( \beta \)), 7.37 - 8.12 (2 x PhCO\( _2 \)) ppm. Chromatography and recrystallisation from methanol gave the pure (99e) as colourless needles. Direct application of the benzylation method to (98, \( R = \text{NH-C}H\_2\text{Ph} \)) gave the mono-protected (99f, 68 %) in good yield.

The benzoyl group was considered the most favourable protecting group as it produced high yields of crystalline mono-protected product and it could easily be removed later in the synthesis by base catalysed hydrolysis.

Steroidal alcohols have been oxidised with a great variety of reagents in the past, particularly with Cr(VI) reagents\(^{126} \) such as Jones’ reagent.\(^{127,99} \) The rate of Jones’ oxidation is so fast,\(^{128} \) that it is often possible to run the reaction as a titration to an end point, the red-coloured chromium VI in the acetone layer indicating complete oxidation. The reaction is relatively specific for alcohols since the acetone solvent prevents further oxidations of the product ketone through its preferential reaction with excess oxidant.

TBDMS ethers have been reported to be stable to Jones’ reagent between 0 and \(-30^\circ \text{C} \).\(^{129} \) However when (99c, Table 4) was subjected to Jones’ oxidation at 0 °C for ten minutes C-3 deprotection occurred and a subsequent oxidation of both C-3 and C-12 hydroxyls to the diketone (147, 67 %, Table 5) was observed. Reduction of reaction temperature and time, to -20 °C and 7 minutes respectively, resulted in no reaction. Subsequently, using an intermediate temperature (-10 °C) and longer reaction time (25 minutes) afforded the desired product (100a, 42 %, Table 5) with a characteristic shift in the \( ^{13} \)C NMR spectrum for C-12 from \( \delta _C \) 73.19 ppm to 212.70 ppm corresponding to the change from hydroxyl to ketone.

Application of these conditions to (99d, Table 4) gave a mixture of desired product (100b, Table 5) and a substantial amount of deprotected and / or oxidised products along with benzoic acid. Chromatography proved time consuming. However, it gave the desired ketone as white prisms after recrystallisation (100b, Table 5, 45 %) from methanol.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Temp. (°C)</th>
<th>Solvent</th>
<th>Time (min.)</th>
<th>Yield</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>(99c)</td>
<td>0</td>
<td>Acetone</td>
<td>10</td>
<td>67%</td>
<td><img src="147" alt="Product" /></td>
</tr>
<tr>
<td>(99c)</td>
<td>-20</td>
<td>Acetone</td>
<td>7.5</td>
<td>-</td>
<td>Starting material</td>
</tr>
<tr>
<td>(99c)</td>
<td>-10</td>
<td>Acetone</td>
<td>25</td>
<td>42%</td>
<td><img src="100a" alt="Product" /></td>
</tr>
<tr>
<td>(99d)</td>
<td>-10</td>
<td>Acetone</td>
<td>20</td>
<td>45%</td>
<td><img src="100b" alt="Product" /></td>
</tr>
</tbody>
</table>

**Table 5 - Oxidations with Jones' Reagent**

The result of oxidations of both (99c) and (99d) showed that the protecting groups at C-3 are prone to acid hydrolysis and that Jones' reagent may have been too harsh a reagent for oxidation.

These results prompted investigation of dipyridinium chromium oxide\textsuperscript{130} in pyridine as a basic oxidising agent. Collins, Hess and Frank\textsuperscript{131} found that a crystalline form of the anhydrous chromium trioxide-dipyridine complex was soluble in chlorocarbon solvents, particularly dichloromethane. By utilising the reagent in this solvent system, they were able to obtain high yields of aldehydes and ketones from corresponding primary and secondary alcohols at room temperature with short reaction times.

Oxidation of (99d, Table 4) occurred under standard Collins conditions to afford the desired product (100b, 48%) after purification via flash chromatography. One unfortunate aspect of using this chromium trioxide-dipyridine based reagent is that six equivalents of oxidant are needed to ensure the rapid and complete oxidation, complicating the work-up. It is
apparent that this requirement for excess oxidant is due, at least in part, to the production of water during the course of oxidation (Scheme 13).

\[
(99d) + \text{CrO}_3.2\text{py} \longrightarrow (100b) + \text{CrO}_2.2\text{py} + \text{H}_2\text{O}
\]

\[
\text{CrO}_3.2\text{py} + \text{H}_2\text{O} \longrightarrow \text{CrO}_7^{2-}(\text{pyH}^+)_2
\]

insoluble

Scheme 13

Collins showed that the CrO\textsubscript{3}.2py reacts rapidly with water to produce the insoluble pyridinium dichromate. The basic CrO\textsubscript{2}.2pyr species is likely to coordinate with and deactivate further quantities of CrO\textsubscript{3}.2pyr.

Various other oxidations were investigated including the use of PCC (Pyridinium chlorochromate) and the Swern oxidation\textsuperscript{132} (DMSO plus oxalyl chloride), but the yield of ketone (100b) was not improved.

PCC\textsuperscript{133} was chosen as although it has a slightly acidic character, some acid labile groups such as tetrahydropyranyl ethers survive the oxidation. However, oxidation of (99d) gave only (100b) in 12 % yield.

Oxalyl chloride in combination with DMSO has been shown to oxidise steroidal alcohols with great success under mild conditions, but with (99d) only a poor yield (17 %) of the ketone (100b) was obtained. Oxidation depends upon the formation of the activated intermediate (149) from spontaneous loss of carbon monoxide and carbon dioxide from (148, Scheme 11).

\[
\begin{align*}
\text{SOCl}_2 + \text{CH}_2\text{Cl}_2 & \xrightarrow{\text{Low temp.}} \text{(148)} \\
\text{(148)} & \xrightarrow{-\text{CO}_2} \text{(149)} \\
\text{(149)} & \xrightarrow{-\text{CO}} \text{(149)}
\end{align*}
\]

Scheme 14
Two-phase chromic acid oxidation has found application in the steroidal field where there is considerable difference in solubility of the starting alcohol relative to the product ketone in the aqueous phase.\(^{134}\) This method can be useful if the product ketone is sensitive to secondary reactions such as oxidation, dehydration or acid hydrolysis. Using diethyl ether as the second phase, \((99d)\) was oxidised cleanly to \((100b, 86\%)\) The mechanism of oxidation with chromic acid has been the object of much study (the mechanism of which was postulated by Westheimer).\(^{135}\)

The intermediate formation of a chromate ester has been observed\(^{136}\) in the past. The hydrogen removal from the \(\alpha\)-position is generally thought of as a cyclic mechanism involving the chromate ester \((150)\) and is generally favoured by chemists (Scheme 15).

![Scheme 15](image)

**Scheme 15**

<table>
<thead>
<tr>
<th>Oxidation Type</th>
<th>Conditions</th>
<th>Isolated Yield of ((100b))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jones</td>
<td>-10 °C, acetone, 30 min</td>
<td>45 %</td>
</tr>
<tr>
<td>Collins</td>
<td>r.t., (\text{CH}_2\text{Cl}_2), 30 min</td>
<td>47 %</td>
</tr>
<tr>
<td>PCC(^{137})</td>
<td>r.t., (\text{CH}_2\text{Cl}_2), 6 h</td>
<td>12 %</td>
</tr>
<tr>
<td>Swern(^{132})</td>
<td>-10 °C, ((\text{COCl})_2\text{CH}_2\text{Cl}_2), 15 min, then allowed to warm to r.t.</td>
<td>17 %</td>
</tr>
<tr>
<td>Two phase Chromate(^{138})</td>
<td>r.t., aq. Chromic acid, (\text{Et}_2\text{O}), 90 min</td>
<td>86 %</td>
</tr>
</tbody>
</table>

**Table 6 - Summary Oxidations of \((99d)\)**
Application of the two-phase oxidation to the benzylamine derivative (99f, Table 4, page 62) led to the formation of (100c, 76%).

As (100b) was obtained in the highest yield this candidate was chosen for further modification (Scheme 3, Section 2.2).

Direct insertion of a nitrogen functionality at C-11 by nitrosation was thought to be applicable to (100b), as the ketone was now in place at C-12. Sheehan and Erman described the preparation of 2-oximinocholestan-3-one (151) by treatment of cholestanone with one equivalent of both n-butyl nitrite and potassium t-butoxide as catalyst.

\[ \text{Scheme 16} \]

The nitrosation reaction involves the abstraction of a hydrogen from the carbon α to the electron withdrawing carbonyl group. The initial product is always the C-nitroso compound, but these are only stable when there is no tautomerizable hydrogen. When there is, the product is the more stable oxime. Under standard conditions, t-butyl alcohol with potassium and isoamyl nitrite, (100b) did not yield the desired product (101, Scheme 4, Section 2.2). Instead, base-catalysed deprotection at C-24 and C-3 occurred (\(^{1}H\) NMR spectrum - no methyl ester peak at \(\delta\) 3.66 ppm or CH-3β at 5.82 - 5.23 ppm), with no signs of incorporation of the nitroso group at C-11.

Changing to refluxing with boiling alkyl nitrites, n-propyl nitrite (bpt. 46 - 49°C) or n-butyl nitrite (bpt. 78°C) in ethanol with sodium ethoxide also proved unsuccessful, producing only deprotected products. The protocol in changing solvent was to prevent complications involved in the work-up and different alkyl nitrites would allow for various activities of
nitrite. Therefore, this route (steps i, x, vii, ix, Scheme 4, Section 2.2) to (86) from (100) was discontinued as no incorporation of nitrogen at C-11 occurred.

Seebeck and Reichstein$^{104}$ and Gallagher and Long$^{124}$ previously reported the bromination of 12-keto-3α-acetoxycholanic acid (Br$_2$/AcOH/r.t./dark/5 days) to give a mixture of 11α- and 11β-epimers, in which the 11α-epimer predominates. Direct application of these conditions to (100b) gave no reaction over five days. Addition of a catalytic amount of HBr resulted in successful bromination at C-11. However, due to the acidic conditions involved, de-esterification occurred at C-24. Re-methylation using diazomethane and purification by chromatography gave (102a) in 29% yield over two steps, as a cream solid. Increasing the temperature of bromination from 20°C to 60°C increased the yield of (102a) to 73% after recrystallisation. The $^1$H NMR spectrum showed C11-H as a doublet at $\delta_H$ 4.95 ppm with an axial-axial coupling (11 Hz) to CH-9 indicating the bromine atom is on the less hindered α face. If the bromine atom had been on the β-face the CH-11 - CH-9 coupling would be axial-equatorial ($J_{\alpha,\alpha}$ in the range 2 - 6 Hz$^{141}$).

No 11β - bromo isomer was isolated or detected. This would be expected to be the kinetic product as suggested by Corey$^{142}$ based upon consideration of orbital overlap in the enol form. The maximum overlap in the transition state is only possible with axial approach in the case of a chair-form ring. Equilibration to the thermodynamic 11α - bromo product would be expected and has been reported by Corey$^{143}$ in the 3α-acetoxycholanic acid series. The presence of acid, including that generated during the course of the reaction can cause rearrangement of the initially formed 11β - bromo product via another enolisation to the 11α - bromo product (102a). (This rearrangement is promoted by the strong steric interaction of C18 and C19 angular methyls).
Following Gallagher's work on the synthesis of cortisone, the 11-bromo-12-keto cholanic acid (152) was treated with strong base to give the four possible ketols (153a, 153b, 154a, and 154b, Scheme 17).

When directly applied to (102a), by refluxing with KOH in ethanol for one hour, the ketol formation took place and, not surprisingly, hydrolysis of the esters occurred. The \(^1\)H NMR spectrum showed no methyl ester singlet at \(\delta_H 3.66\) ppm or aromatic protons at 7 - 8 ppm. Subsequent re-methylation and dibenzoylation of the C3-OH and the C11-OH or the C12-OH gave a mixture of isomers which were separated by preparative thin layer chromatography to give compounds (155, 19 %), (156a, 32 %) and (156b, 19 %) (Scheme 18).

The \(^1\)H NMR spectrum of (155) reveals a doublet (\(J = 11\) Hz) at \(\delta_H 5.73\) ppm with similar characteristics to the \(\alpha\)-bromoketone (102a) including axial-axial coupling between C11-H and C9-H. For (156a) and (156b) the stereochemistry around C12 was deduced by difference nOe (nuclear Overhauser effect) experiments. By irradiating the C12 methine proton at...
δ_H 5.23 ppm (CH-12α, 156a) and 5.38 ppm (CH-12β, 156b), difference spectra were taken and compared with original ^1^H NMR spectra (Fig. 47).

H atoms in red are the protons which have been irradiated. H atoms in blue are the protons affected by the irradiation. % are with respect to the integration of peak irradiated, designated 100%.

Fig. 47

The proportions of isomers reflect the differences in stability of ketols, arising from non-bonded interaction. The main feature is the preference for the C11 position for the oxo functionality, especially with regard to (156a). This avoids the large diaxial interactions, especially with the C13 and C10 methyls. The mechanism of the reaction is thought to be S_{x2} to (157, Scheme 19) followed by equilibration (through rearrangement) of the various ketols (Scheme 19).

Scheme 19
Enolisation of the oxo-function could give the ene-diol intermediate (158) and ketonisation may then give either the original ketol (157, Scheme 19) or the rearranged ketol (159, Scheme 19). An alternative mechanism (Scheme 19, dotted line) may involve an intramolecular hydride transfer.\(^{145}\)

As the correct \(\beta\)-stereochemistry for the protected hydroxyl group at C12 (156a) was now in place, introduction of a nitrogen functionality was attempted at C11 through the ketone as originally proposed (step vi, Scheme 4, Section 2.2).

Two general methods exist for the formation of oximes.\(^{146}\) However, only the more common method which relies upon the treatment of an aldehyde or ketone with the salt of hydroxylamine was relevant to our investigation. Hydroxylamine and its derivatives, which are sensitive and decompose in their free form, are supplied as their salts, which can then be completely, or partially neutralised by the addition of base or by carrying out the reaction in pyridine.\(^{147}\) The reaction between hydroxylamine and a carbonyl group reflects the similarities in the mechanism of carbonyl condensations with nitrogen bases (Scheme 20).

\[
\begin{align*}
R^1\text{R}^2\text{C}=O &+ \text{H}_2\text{NR}^3 \rightarrow \text{fast} \quad \overset{\text{slow}}{\leftarrow} \quad \overset{\text{fast}}{\leftrightarrow} \\
(160) &\quad \overset{\text{fast}}{\leftrightarrow} \quad (161) &\quad \overset{\text{slow}}{\rightarrow} \quad (162) &\quad \overset{\text{slow}}{\leftrightarrow} \quad (163) \\
\end{align*}
\]

**Scheme 20 - Proposed Mechanistic Pathway for the Condensation Reaction between Nitrogen Bases and Carbonyl Compounds.**

In the first step, the nitrogen base adds to the carbonyl compound (160) to give the carbinolamine intermediate (162), followed by elimination of water to form the imine bond (163) in the second step. The bimolecular mechanism
has been reviewed exhaustively\textsuperscript{148} and shows that the pH of the reaction medium is critical. The rate of formation of oximes is at a maximum at a pH which depends on the substrate (usually about 4), and the rate decreases as the pH is either raised or lowered from this point.

The relative unreactivity of 11-ketosteroids towards the usual ketonic reagents is well documented,\textsuperscript{149,150} however, 11-oximino steroids have been prepared.\textsuperscript{151,152} Oximation of (156a and 156b) was attempted with various conditions as shown in Table 7.

<table>
<thead>
<tr>
<th>Starting Material</th>
<th>Solvent</th>
<th>Reaction Conditions</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>(156a)</td>
<td>Ethanol</td>
<td>NH\textsubscript{2}OH.HCl, r.t., 7 days</td>
<td>no reaction</td>
</tr>
<tr>
<td>(156a)</td>
<td>Pyridine/H\textsubscript{2}O</td>
<td>NH\textsubscript{2}OH.HCl, reflux, 7-14 days</td>
<td>no reaction</td>
</tr>
<tr>
<td>(156a)</td>
<td>Pyridine/H\textsubscript{2}O</td>
<td>NH\textsubscript{2}OSO\textsubscript{3}H\textsubscript{,} reflux, 7-14 days</td>
<td>no reaction</td>
</tr>
<tr>
<td>(156a)</td>
<td>Pyridine/H\textsubscript{2}O</td>
<td>NH\textsubscript{2}O\textsubscript{butyl}.HCl, reflux, 7-14 days</td>
<td>no reaction</td>
</tr>
<tr>
<td>(156b)</td>
<td>Pyridine/H\textsubscript{2}O</td>
<td>NH\textsubscript{2}OH.HCl, reflux, 7-14 days</td>
<td>no reaction</td>
</tr>
</tbody>
</table>

Table 7

Standard conditions of hydroxylamine hydrochloride in ethanol, were first attempted, but no reaction occurred. A combination of altering the solvent to pyridine, the temperature (0 - 115 °C), the reaction times (up to 14 days) and the hydroxylamine salt used all proved unsuccessful.

High yields of oximes (successful for hindered ketones) have been obtained when the ketone is allowed to stand with hydroxylamine and a strongly basic catalyst for up to six months.\textsuperscript{153} However, due to time constraints this method would not have been viable.

Severe non-bonded interactions with the angular methyl groups at C10 and C13, and the bulky C-12β-benzoate strongly hinder the formation of the tetrahedral carbinolamine (162, Scheme 20) believed to be formed in

73
Due to these steric difficulties, this pathway (steps iv, vi, vii, ix, Scheme 4) was not carried on any further as the hydroxy oxime (106, Scheme 4, Section 2.2) could not be synthesised.

The focus of the synthesis then turned towards the use of the α-bromo-ketone intermediate (102, Scheme 4). In attempts to prepare 2α-azidocholestanone (165, Scheme 21) from the corresponding α-bromoketo compound (164, Scheme 21), Edwards and Purushothaman\textsuperscript{154} found that the reaction of 2α-bromocholestanone (164, Scheme 21) with lithium azide in methanol or sodium azide in dimethyl sulphoxide proved unsuccessful. A steady state concentration of the azidoketone (165, Scheme 21) was produced as revealed by IR spectroscopy ($\nu_{\text{max}}$ 2110 and 1655 cm$^{-1}$) but after one hour at 50 °C with excess lithium azide, the bromoketone had been completely converted to an α-iminoketone (166, Scheme 22), proved by hydrolysis to the dione, reduction and then acetylation to the 2-aminocholestan-3-ol which was characterized as its O,N-diacetate.

\[
\text{Scheme 21}
\]

Direct application of this $S_N2$ displacement to our α-bromoketone (102a) using excess sodium azide in anhydrous dimethylsulphoxide at 100 °C for 48 hours gave a compound detected by TLC chromatography (blue spot obtained with ninhydrin (primary amine)). Chromatography afforded a colourless foam, $\nu_{\text{max}}$ 3512 and 3367 cm$^{-1}$ (unsymmetrical and symmetrical NH stretching) and 1685 cm$^{-1}$ ($\alpha,\beta$-unsaturated ketone) corresponding to an
enaminoketone (168, Scheme 22, 53%) and not the predicted α-azidoketone (167, Scheme 22). Attempts at recrystallisation from various solvents and hydrochloride salt formation proved unsuccessful.

Although azides have generally been used to form amines, α-keto azides are known to decompose relatively easily to form imino ketones or enaminoketones in the presence of azide ion on treatment with base.155,156,157 The azido ketone (167) would have an 11β-axial azide group which would be sterically congested (as shown in Fig. 48) by significant interaction with the angular methyl groups. The relief of steric interactions between the axial methyl groups (C18 and C19) and the azido group appeared to be a conformational driving force for the decomposition and loss of nitrogen from the azidoketone (167, Scheme 23).

Scheme 22

Scheme 23
Azide ion probably acted as a generalised base aiding the abstraction of C11-H then tautomerisation to (168, Scheme 23). Although the enamine (168, Scheme 23) is prone to hydrolysis, it could be stored under nitrogen for limited periods in the freezer. The 12-keto group would be expected to account for its reasonable stability compared to other simple enamines.

Attempts to increase the yield of enamine (168, Scheme 23) or the azide (167, Scheme 23) using trimethylsilyl azide in dichloromethane, sodium azide in DMF, methanol or ethylene glycol at various temperatures (50 - 150 °C), and lithium azide in DMF, methanol or ethylene glycol at various temperatures (50 - 150 °C) all proved less successful than sodium azide in DMSO.

On one occasion, subjecting the crude α-bromo ketone (102a) to excess sodium azide in anhydrous dimethyl sulphoxide (100 °C, 48 hours) gave the enamine (168) as usual, with a second compound, deduced to be the azido-ketone (170, Scheme 24, 4%), after chromatography. The 1H NMR spectrum of (170) revealed a doublet at δH 5.00 ppm (J = 11 Hz) similar to that of
(102a, Scheme 22) and (155, Scheme 18) and Infrared spectrum showed a sharp peak at \( \nu_{\text{max}} \) 2103 cm\(^{-1}\) characteristic of an azide.

\[
\begin{align*}
\text{(170)} && \xrightarrow{\text{H}_2/\text{Pd/C, EtOH, r.t.}} && \text{(171)}
\end{align*}
\]

**Scheme 24**

Hydrogenation of azides occurs with retention of configuration.\(^{159}\) When the azido ketone (170, Scheme 24) was reduced with hydrogen and 5 % palladium on charcoal a shift upfield of \( \delta_H \) 1.4 ppm (\( J_{ax,ax} = 11 \) Hz) for C11-\( \beta \)H in the \(^1\)H NMR spectrum, coupled with peaks in the IR spectrum at \( \nu_{\text{max}} \) 3459, 3358 cm\(^{-1}\) (unsymmetrical and symmetrical NH stretching) and 1602 cm\(^{-1}\) (amine) suggested the formation of amino ketone (171, Scheme 24). As only very small amounts of both azido ketone (170) and amino ketone (171) were available, no further transformations were attempted upon these two compounds.

The formation of azido ketone (170, Scheme 24) and reduction to amino ketone (171, Scheme 24) implied either 11\( \beta \)-bromo-12-ketone (102b) was present in the crude mixture subjected to S_n2 azide displacement or re-enolisation of \( \beta \)-azido ketone (167) occurs to the more stable \( \alpha \)-azido ketone (170).

As a nitrogen function had now been introduced into C11, appropriate protection of the enamine (168) with a 'bulky' hydrophobic group was required to comply with earlier molecular modelling studies (Chapter 1).

Many protecting groups have been developed for an amino group, including carbamates (>NC\(_2\)R), used mainly for the protection of amino acids and in protein syntheses, and amides (>NCOR) used more widely in the syntheses of alkaloids.
The tert-butoxycarbonyl group (Boc) is one of the most frequently used amino protecting groups in organic synthesis. The Boc group is completely stable to catalytic hydrogenolysis conditions (and reducing agents generally) and extremely resistant towards basic and nucleophilic reagents making it an ideal protecting group for the introduction of the correct sterochemistry around C11, C12 and aromatisation of the A-ring (steps i-vi, Scheme 6, Section 2.4).

The instability of tert-butyl chloroformate precludes its use for preparing Boc derivatives and so a large number of alternative reagents and methods have been developed of which Boc₂O\(^{160}\) (di-tert-butyl dicarbonate) and 2-(tert-butoxycarbonyloxyimino)-2-phenylacetonitrile\(^{161}\) ("Boc-ON") are the most commonly used.

\[
\text{PhCO}_2\text{H} + \text{BOC}OO\text{H}_2\text{Cl}_2, \text{reflux, or} \\
\text{PhCO}_2\text{H} + \text{BOC}OO\text{H}_2\text{EtOH}, \text{reflux, or} \\
\text{PhCO}_2\text{H} + \text{NEt}_3, \text{toluene, reflux}
\]

\[
\begin{align*}
\text{Boc-O-N=C(CN)Ph, NEt}_3, \text{EtOH, reflux} \\
\text{Boc-O-N=C(CN)Ph, NEt}_3, \text{toluene, reflux}
\end{align*}
\]

Scheme 25

Incorporation of the Boc protecting group into enamine (168) was unsuccessful when using either Boc₂O or Boc-ON (Scheme 25). Changing the solvent and strength of base appeared to have no effect upon the reaction.
N-Carbamates are typically formed from reaction of an amine with a wide variety of reagents of which using a chloroformate in the presence of a base (such as K$_2$CO$_3$, DMAP or NEt$_3$) is the most common.

\[ \text{R-CI, DMAP, CH}_2\text{Cl}_2, \text{reflux, 18hr} \]

\[ \text{PhCO}_2^- \text{NH}(H) \rightarrow \text{PhCO}_2^- \text{R-NH} \]

(168) \quad (173a, 27\%, R = C_2H_5OCO-)  
(173b, 27\%, R = Cbz-)  
(173c, 46\%, R = (CH_3)_3CCO-)

\textbf{Scheme 26}

Ethoxycarbonyl derivatisation is one of the simplest forms of carbamate-type protecting groups in common use since 1903.$^{162}$ Protection of enamine (168) with ethoxycarbonyl chloroformate and DMAP gave the ethoxycarbonyl derivative (173a, 27\%, Scheme 27) purified by preparative TLC. The structure of (173a) was supported by the $^1$H NMR spectrum; $\delta$$_H$ 1.2 ppm (triplet, $J$ = 7 Hz, CH$_2$CH$_3$) and 4.1 ppm (multiplet, CH$_2$CH$_3$) characteristic of an ethyl group, and urethane carbonyl peak in the $^{13}$C NMR spectrum at $\delta$$_C$ 156.06 ppm.

Introduced by Bergmann and Zervas$^{163}$ in 1932 for the protection of amino groups in peptide synthesis, the reagent Cbz-CI still affords probably the most widely used means of N-protection. Protection of enamine (168) with Cbz-CI and DMAP occurred smoothly to give the Cbz derivative (173b, 27\%, Scheme 27) after purification by preparative TLC.

The $^1$H NMR of (173b) showed line broadening at 20 °C characteristic of certain 'bulky' carbamates.$^{164}$ Variable temperature $^1$H NMR from 25 °C to 95 °C showed several conformational changes and sharpened up the spectra.
Fig. 49 - Variable temperature $^1$H NMR of (173b)

25 - 55 °C (CDCl$_3$, bottom), 65 - 95 °C ($d_6$ - DMSO, top)
As can be seen from the variable temperature spectra at 25 °C (CDCl₃) only broad peaks may be seen for the C18, C19 and C21 methyl peaks suggesting that (173b) exists in solution as two or more conformers. As the temperature is increased to 55 °C (CDCl₃) a doublet (J = 6 Hz) at δH 0.98 ppm and a singlet at 1.15 ppm start to emerge corresponding to the methyls C21 and C19 in the final averaged conformation at 95 °C (d₆-DMSO) respectively. At 95 °C (d₆-DMSO), ¹H NMR showed sharpened spectra of the averaged conformers, C18, C19 and C21 methyls appearing as singlet (δH 0.87 ppm), singlet (δH 1.24 ppm) and doublet (δH 0.85 - 0.90 ppm) respectively. A shift upfield for the C19 methyl can be seen from δH 1.00 to 0.93 ppm when the solvent was changed from CDCl₃ to d₆-DMSO.

As the anticipated Boc derivative was unobtainable by methods previously discussed (Scheme 25), the N-pivaloyl (trimethylacetyl) was the next target to provide a large hydrophobic group on the nitrogen in accordance with most potent HIV-1 protease inhibitors (as discussed in Chapter 1).

In an early study of mixed anhydride peptide synthesis, Vaughan and Osato¹⁶⁵ mentioned the pivaloyl chloride (trimethylacetyl chloride) as a protecting reagent following studies by Zaoral¹⁶⁶ and Kenner et al.¹⁶⁷ Standard protection conditions for the synthesis of N-pivaloyl protected amines (trimethylacetyl chloride, DMAP, dichloromethane, reflux, 16 hours) were attempted on enamine (168) yielding the pivaloyl-protected enamine (173c, 46 %) after preparative TLC. Incorporation of t-butylcarbonyl was verified by the NMR spectra; δH 1.22 (singlet) ppm, 27.29 (t-butyl) and δC 179.11 (C=O) ppm in the ¹H and ¹³C NMR respectively.

In order to follow the original pathway (Scheme 4, Section 2.2) to the original targets (86) through (107), protected enamines (150a, 150b, 150c) must be reduced at Δ⁹,¹¹ and 12-ketone.

Catalytic hydrogenation has been used extensively in steroid research, and the method has been found to be of great value for the selective and
stereospecific reduction of olefins and ketones.\textsuperscript{168} As a result of extensive work on hydrogenation of olefins,\textsuperscript{169,170} the mechanism originally proposed by Horiuti and Polanyi\textsuperscript{171} is currently accepted.

Most steroid hydrogenations are performed on palladium, platinum or Raney nickel. The reaction involves adsorption of hydrogen and the olefin bonds onto 'active centres' on the catalyst surface, where the hydrogen molecule is at least partially dissociated to give reactive hydrogen atoms bonded to the catalyst and the olefin becomes attached by two \(\sigma\)-type bonds to adjacent 'active centres' before accepting two hydrogen atoms in discrete steps from the neighbouring catalyst surface. The resulting saturated molecule, being held to the surface only by weak Van der Waals forces, is rapidly desorbed. The mechanism has the stereochemical consequence that hydrogen addition is selectively cis.

Because of the shape and rigidity of the steroid ring system, double bonds at various positions are hydrogenated with different degrees of facility. \(\Delta^4, \Delta^8, \Delta^9,11, \Delta^7\) and \(\Delta^8,14\) double bonds are progressively more difficult to hydrogenate than \(\Delta^5\) with the \(\Delta^8,14\) olefin inert to hydrogenation except under drastic conditions.

The conjugation of an olefin with a carbonyl group can markedly increase the susceptibility of the former group to hydrogenation. Since ketones are generally inert to hydrogenation over palladium, this catalyst is preferred for the selective hydrogenation of the double bond in \(\alpha,\beta\)-unsaturated ketones such as (173a, 173b, 173c). In accordance with the work of McKenzie et al\textsuperscript{172} upon 12-keto-\(\Delta^9,11\)-cholic esters, hydrogenations of protected enamines (173b), (173c) were attempted with various conditions. However, using 10 \% palladium upon charcoal / MeOH / room temperature / 1 atmos. \(H_2\) and both proved resistant to hydrogenation and no change in starting material was observed over five days (Table 8).

The effect of hydrogen pressure on the rate of the reaction is non-linear and dependent on the nature of the steroid and the catalyst used.\textsuperscript{173} An increase
in hydrogen pressure may also result in a decrease in reaction selectivity. For those compounds having more than one reactive group, the relative rates of hydrogenation of these entities are brought closer together with increasing pressure and it becomes more difficult to stop the reaction at the desired stage of completion, particularly with the large amount of hydrogen present at the higher pressures.

Attempted hydrogenations of protected enamines (173b) and (173c) and unprotected enamine (168) with 10% Pd upon charcoal under 10 atmos. H₂ proved unsuccessful.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Catalyst</th>
<th>Pressure</th>
<th>Solvent</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>(173b), (173c)</td>
<td>10 % Pd / C</td>
<td>1 atmos</td>
<td>MeOH</td>
<td>20 °C</td>
</tr>
<tr>
<td>(173b), (173c), (168)</td>
<td>10 % Pd / C</td>
<td>10 atmos</td>
<td>MeOH</td>
<td>20 °C</td>
</tr>
<tr>
<td>(173b), (173c)</td>
<td>10 % Pd / C</td>
<td>1 atmos</td>
<td>EtOH</td>
<td>20 °C</td>
</tr>
<tr>
<td>(173b), (173c)</td>
<td>10 % Pd / C</td>
<td>1 atmos</td>
<td>EtOAc</td>
<td>20 °C</td>
</tr>
<tr>
<td>(173b), (173c)</td>
<td>10 % Pd / C</td>
<td>1 atmos</td>
<td>EtOH</td>
<td>50 °C</td>
</tr>
<tr>
<td>(173b), (173c)</td>
<td>10 % Pt / C</td>
<td>1 atmos</td>
<td>EtOH</td>
<td>20 °C</td>
</tr>
<tr>
<td>(173b), (173c)</td>
<td>10 % Pt / C</td>
<td>10 atmos</td>
<td>EtOH</td>
<td>20 °C</td>
</tr>
<tr>
<td>(173b), (173c)</td>
<td>10 % Pt / C</td>
<td>1 atmos</td>
<td>EtOH</td>
<td>50 °C</td>
</tr>
<tr>
<td>(173b), (173c)</td>
<td>PtO₂</td>
<td>1 atmos</td>
<td>EtOH</td>
<td>20 °C</td>
</tr>
<tr>
<td>(173b), (173c)</td>
<td>PtO₂</td>
<td>10 atmos</td>
<td>EtOH</td>
<td>20 °C</td>
</tr>
<tr>
<td>(173b), (173c)</td>
<td>PtO₂</td>
<td>1 atmos</td>
<td>EtOAc</td>
<td>20 °C</td>
</tr>
<tr>
<td>(173b), (173c)</td>
<td>PtO₂</td>
<td>1 atmos</td>
<td>EtOH</td>
<td>50 °C</td>
</tr>
<tr>
<td>(173b), (173c)</td>
<td>PtO₂</td>
<td>1 atmos</td>
<td>EtOAc/ HOAc</td>
<td>20 °C</td>
</tr>
<tr>
<td>(173b), (173c)</td>
<td>(Ph₃P)₃RhCl</td>
<td>1 atmos</td>
<td>EtOH</td>
<td>20 °C</td>
</tr>
</tbody>
</table>

[1 atmos. H₂ (Hydrogen filled balloon), 10 atmos. H₂ (Parr Hydrogenator).]

Table 8 - Attempted Hydrogenations of Protected (173b), (173c) and Unprotected (168) Enamines.
Generally, the stereochemistry of the products obtained on hydrogenation of steroidal olefins is not affected by the nature of the solvent. Changing the reaction medium to ethanol or ethyl acetate also proved unsuccessful in performing catalytic hydrogenation upon (173b) and (173c).

The effect of temperature on the rate of the reaction has been studied for a variety of hydrogenations. The exact effect observed is dependent on the nature of the substance being hydrogenated and on the type of catalyst being used, but for almost all hydrogenations the rate will approximately double for every 10 - 20 °C increase in temperature. The increase of temperature from 20 °C to 50 °C showed no augmentation in the reactivity of (173b) or (173c) with a range of catalysts (Table 8).

Platinum is capable of promoting the hydrogenation of most functional groups under relatively mild conditions, usually at temperatures below 70 °C. Platinum supported upon charcoal has very similar catalytic activity to palladium upon charcoal. Esters, carboxylic acids and most amides are the only common functional groups not hydrogenated over platinum, which would make platinum upon charcoal highly selective for the reduction of the \( \Delta^{9,11} \) double bond. Unfortunately, hydrogenation did not take place on compounds (173b) and (173c) under standard conditions or when varying solvent, temperature or pressure.

The usual form of platinum catalyst is the oxide PtO\(_2\) (Adams' Catalyst). The popularity of Adams' Catalyst stems from the fact that a spent catalyst can be reconverted to the oxide, thus producing a method for the recycling of the platinum. The \( \Delta^{9,11} \)-12-keto steroid (174, Scheme 27) was hydrogenated over platinum oxide in acetic acid, illustrating the inertness to hydrogenation exhibited by the \( \Delta^{6,11} \) double bond (Scheme 27). Surprisingly, reduction of the carbonyl group of \( \Delta^{9,11} \)-12-ketone takes place prior to the saturation of the double bond.
Hydrogenations of $\alpha,\beta$-unsaturated ketones in acidic media involves 1,4-hydrogen addition via a protonated form of the ketone, which exhibits greater selectivity in bonding to the catalyst than does the non-polarised compound in neutral solution, producing the allylic alcohol ($175$). Application of Adams' catalyst in acidic solvents to ($173b$) and ($173c$) did not hydrogenate the 12-keto moiety or the $\Delta^{8,11}$-double bond. However, some ester removal was observed at C-24 by acid hydrolysis.

In the past the homogeneous hydrogenation catalyst ($\text{Ph}_3\text{P})_3\text{RhCl}$ has been used for the reduction of steroidal double bonds. $\Delta^1$, $\Delta^2$, and $\Delta^3$. Double bonds are readily saturated, but isolated $\Delta^4$- or $\Delta^5$-olefins are inert to this catalyst.

The $\Delta^5$ double bond of ergosterol ($176$) is reduced selectively to give the $5\alpha$-$\Delta^7$-product ($177$, Scheme 28), as is the case with heterogeneous catalysis, hydrogen entering exclusively from the $\alpha$-face. The mechanism of homogeneous hydrogenation catalysed by ($\text{Ph}_3\text{P})_3\text{RhCl}$ involves reaction of the catalyst with hydrogen to form a metal hydride, which rapidly transfers two hydrogen atoms to the alkene.
Although the addition is syn, there is evidence that the actual addition takes place in a stepwise fashion.\textsuperscript{161} We found that attempts to repeat conditions of Djerassi and Gutzwiller to reduce the $\Delta^{9,11}$-double bond in (173b) and (173c) proved unsuccessful.

Previously, $\Delta^{9,11}$-double bonds are hydrogenated almost exclusively from the $\alpha$-face regardless of the substitution elsewhere in the molecule.\textsuperscript{162,183} However when hydrogenation with a number of catalysts and conditions upon (173b) and (173c) were attempted no reaction took place.

This lack of reactivity to hydrogenation with various catalysts gave only starting material, perhaps not surprisingly in view of other observations on $\Delta^{9,11}$-12-ketones.\textsuperscript{192} Energy minimisations of (173b) and (173c) were performed upon CAChe\textsuperscript{™} ver. 4.0 Molecular Modelling package and showed the C-ring would be preferably approached from the $\alpha$-face as approach from the $\beta$-face is more hindered by interactions with the angular methyl groups (C18, C19 and C21). While the $\alpha$-face is the more accessible, there is sufficient interaction from the benzoate substituent at C3 and methines at C7, C14 to leave incoming attacking species inactive towards $\Delta^{9,11}$-double bond and/or the 12-ketone functions within the systems of (168), (173a), (173b) and (173c).

Due to the lack of reactivity within these compounds to hydrogenation, an alternative reduction of the C12 ketone before reduction of $\Delta^{9,11}$-double bond was pursued.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Reaction Conditions</th>
<th>Temp. (°C)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>(168)</td>
<td>CeCl₃, NaBH₄, EtOH</td>
<td>0</td>
<td>Intractable mixture</td>
</tr>
<tr>
<td>(173b), (173c)</td>
<td>CeCl₃, NaBH₄, EtOH</td>
<td>0</td>
<td>C-11 Deprotection</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C-24 Deprotection</td>
</tr>
<tr>
<td>(173b) and (173c)</td>
<td>CeCl₃, NaBH₄, EtOH</td>
<td>78</td>
<td>C-24 Deprotection</td>
</tr>
<tr>
<td>(168)</td>
<td>NaBH₃CN, H⁺, THF</td>
<td>20</td>
<td>Intractable mixture</td>
</tr>
<tr>
<td>(173b) and (173c)</td>
<td>NaBH₃CN, H⁺, THF</td>
<td>20</td>
<td>No Reaction</td>
</tr>
<tr>
<td>(173b) and (173c)</td>
<td>9-B.B.N., THF</td>
<td>0</td>
<td>No Reaction</td>
</tr>
<tr>
<td>(173b) and (173c)</td>
<td>9-B.B.N., THF</td>
<td>25</td>
<td>No Reaction</td>
</tr>
<tr>
<td>(173b) and (173c)</td>
<td>K-Selectride, THF, N₂</td>
<td>-20</td>
<td>No Reaction</td>
</tr>
<tr>
<td>(173b) and (173c)</td>
<td>K-Selectride, THF, N₂</td>
<td>20</td>
<td>No Reaction</td>
</tr>
</tbody>
</table>

Table 9 - Attempted Reductions of 12-Ketone Function.

The reduction of organic compounds by complex metal hydrides, first reported in 1947,¹⁸⁴ is a widely used technique. The reduction of steroidal 12-ketones provides an interesting example of reactions apparently controlled mainly by the product stabilities. The product ratio is determined by the nature of the side chain. A cholane or cholestane side chain, in its stable conformation, places the C21 methyl group in steric opposition to a 12β-substituent with a significant increase in conformational strain energy and results in a preference for the formation of the axial 12α-alcohol by complex hydride reduction \((41\alpha:31\beta)^{101}, (4\alpha:1\beta)^{185} \text{ and } (57\alpha:43\beta)^{186}\). These product ratios are at least qualitatively similar to those obtained by metal / alcohol reductions and are the reverse of what one would expect if the steric factors mentioned were to operate specifically against the approach of the complex hydride anion.

In general, the course of a reaction involving the hydride reduction of a ketone is not affected by the presence of additional functional groups in a
molecule. A major exception to this occurs when a ketone is conjugated to a double bond. A conjugated ketone is less reactive than the corresponding unconjugated compound. With sodium borohydride, sometimes reduction of the ketone is accompanied by saturation of the double bond. The reduction of a conjugated ketone by sodium borohydride was first described by Sondheimer and co-workers\textsuperscript{187} and subsequent steroidal reductions followed,\textsuperscript{188,189,190} however some saturation of the double bond was also observed giving many products.

The double bond reduction may be avoided by the use of sodium borohydride in the presence of lanthanide chlorides (LaCl\(_3\), CeCl\(_3\), SmCl\(_3\))\textsuperscript{191} affording almost exclusively the 1,2-addition product (the allylic alcohol). Application of Luche reduction conditions (NaBH\(_4\)/CeCl\(_3\)/EtOH) to some \(\Delta^{9,11}\)-12-keto bile acids has proved successful in the past, giving the allylic alcohol epimers in varying ratios.\textsuperscript{192}

Direct application of Luche conditions using one and two equivalents of sodium borohydride to enamine (168) gave an intractable mixture which showed no amino / imino / azido stretches in crude IR, whereas, protected enamines (173b) and (173c) showed no reaction after one day. Increasing the concentration of sodium borohydride (five equivalents) and varying the reaction time (up to five days) for (173b) and (173c) gave intractable mixtures of C-24 deprotected and compounds devoid of C11 nitrogen substituents by crude NMR and IR, inseperable by preparative TLC (Table 9).

Reduction of the \(\alpha,\beta\)-unsaturated ketone system in (173b) or (173c) to an allylic alcohol, followed by tautomerisation of the enamine to an imine could potentially leave the C-11 imine open to hydrolysis. The hydrolysis of the imine may explain why mixtures devoid of C-11 nitrogen substituents were detected and will be explained later in conjunction with ring opening of epoxides with nitrogen nucleophiles.
An increase in temperature to reflux, as required to reduce certain inert ketones (e.g. 11-keto steroid), was attempted upon (173c) with two equivalents of sodium borohydride in methanol. This provided the C24 deprotected acid (determined by re-methylation to (173c) with diazomethane, Table 9).

Sodium cyanoborohydride (NaBH₃CN) reduces a wide variety of organic functional groups with remarkable selectivity. Even under the acidic reaction conditions employed, many sensitive functional groups are not reduced, such as amides, esters, lactones, nitriles, nitro compounds and epoxides. Sodium cyanoborohydride would potentially reduce the 12-keto group selectively in (168), (173b) and (173c) in the presence of the other functionalities within these molecules. The reduction of aldehydes and ketones is pH dependent, the reaction proceeding at pH 3 - 4, therefore, as the reduction consumes hydrogen ions (Scheme 29), acid must be added to maintain the necessary low pH.

\[
\text{BH}_3\text{CN}^- + 3 \text{ROH} + \text{H}^+ \rightarrow \text{B(OR)}_3 + \text{HCN} + \text{C}^\text{OH}_\text{H}
\]

**Scheme 29**

Even though cholestenone type steroids have shown to give the corresponding allylic alcohol with NaBH₃CN in THF, attempted reductions of (173b) and (173c) showed no reaction over two days, whereas enamine (168) gave an intractable mixture (crude IR similar to that with Luche conditions) inseperable by preparative TLC (Table 9).

Conjugated aldehydes and ketones have been reduced rapidly and quantitatively to the corresponding allylic alcohols by 9-borabicyclo[3.3.1]nonane (9-BBN) in tetrahydrofuran in excellent purities. The mildness of the reducing agent permits the selective reduction of enones in the presence of numerous other functional groups. Application of this reducing agent to our systems was permitted through the
addition of a stoichiometric quantity of 9-BBN added dropwise in THF to (173b) and (173c). The reaction was maintained at 0 °C for six hours and then at 25 °C for one hour but showed no reaction with either steroid (Table 9) proving 9 B.B.N to be too mild a reagent to reduce either the 12-ketone or even the Δ^9,11-double bond.

Cyclic enones have been shown to undergo conjugate addition with K-Selectride (potassium tri-sec-butylborohydride). However, when a β-substituent is present on the enone functionality only 1,2 reduction of the enone was observed but the isomer ratios of the allylic alcohols formed were not determined. Steroidal ketones have been selectively reduced at C3 and C7 and we have subsequently tried to extend this method to the C12 ketone, in an attempt to form the axial 12β-alcohol. Compounds (173b) and (173c) were added dropwise to solutions of K-Selectride in THF at -20 °C under nitrogen. After five hours at -20 °C no change was observed by TLC. After further addition of K-Selectride and extending reaction time to ten hours, allowing the reaction temperature to gradually increase to room temperature, still no change was observed (Table 9). Reduction of rigid cyclic α,β-unsaturated ketones, in the absence of other polar substituents, with the bulky trialkylborohydrides normally yields a mixture of allylic alcohols with the most thermodynamically stable equatorial alcohol predominating. However, due to the steric constraints around the steroidal C-ring in (173b) and (173c), no reaction with K-Selectride took place.

Attempted reductions of either the 12-ketone and / or the Δ^9,11-double bond in enamine (168) and protected enamines (173a, 173b, 173c) using various methods reported in this section gave either no reaction or the formation of complex mixtures. This is possibly due to the inherent instability of the enamine function in the absence of the 12-ketone and / or the steric confinement within the C-ring from the angular methyls. Attempted hydrogenations as reported earlier gave only unreacted starting material, not surprisingly, in view of other observations on Δ^9,11-12-ketone systems.
As stereocentres have proved difficult to introduce into (173b) and (173c) this pathway through enamine (168) was terminated. However, compounds (168, 173a, 173b and 173c) have been evaluated for activity against HIV-1 protease in cell lines and their biological activity will be discussed later in Chapter 4.

Following step iii, Scheme 2, electrophilic amination\textsuperscript{202} of ketone (100) would introduce nitrogen functionality at C-11. Evans\textsuperscript{102} reported that $\alpha$-amino acids are readily obtained by hydrogenolysis of $\alpha$-hydrazino compounds, which are easily accessible in good yields and high optical purity through amination of chiral enolates by dialkyl azodicarboxylates.

Dialkyl azodicarboxylates react with the lithium enolates derived from the N-acyl-oxazolidinones, to provide the hydrazide adducts in excellent yields (Scheme 30), which may be converted to their $\alpha$-amino acid adducts by cleavage with Raney Ni / $H_2$.\textsuperscript{203}

\begin{center}
\begin{align*}
\text{Scheme 30}
\end{align*}
\end{center}

In a typical experimental procedure, N-acyloxazolidones, obtained by N-acylation of oxazolidinones, were converted to their lithium enolates\textsuperscript{204} (1.1 equiv. of LDA, THF, -78 °C) and a solution of dialkyl azodicarboxylate (1.1 equiv., THF, -78 °C) was added. The reactions were then immediately quenched with ammonium chloride solution and conventional isolation procedures afforded the hydrazides as a varying mixture of diastereomers.

Bullman Page and co-workers\textsuperscript{205} extended this electrophilic amination to syn and anti 2-acetyl-2-ethyl-1,3-dithiane 1-oxides with DBAD (di-tert-butyl...
azodicarboxylate) as potential synthons for chiral α-amino acid synthesis. As the introduction of a 'packaged' NH-Boc group (as a protected hydrazide) at C-11 in (100b) would be highly desirable and possibly HIV-1 protease active in their own right, the first choice of reagent for electrophilic amination was DBAD. Since diastereomers are generally difficult to separate, the use of the more hindered DBAD has been found to be synthetically advantageous because, as the size of the alkyl group of the aminating reagent increases the diastereomeric ratio improves.

Under standard Evans' conditions (as stated above), ketone (100b) was reacted with freshly prepared LDA (lithium di-isopropylamide) in THF at -78 °C forming a pale yellow solution. DBAD was added and the solution was stirred for 30 minutes at which point the reaction was then quenched with ammonium chloride to afford only starting material.

Reducing the size of the aminating agent to DEAD (diethyl azodicarboxylate) under standard aminating conditions gave a colour change to pale pink, but on work-up no reaction was observed. Changing the base from LDA to lithium hexamethyldisilazide (LHMDS) with the use of DBAD or DEAD upon (100b) also proved unsuccessful.

In an attempt to determine the ability of C-12 ketone of (100b) to enolise, the ketone was reacted with LDA in THF for 30 minutes at -78 °C which was then quenched with chlorotrimethylsilane to give the corresponding trimethylsilyl enol ether. The crude 1H NMR spectrum of this product revealed a small singlet at δH 4.51 ppm which corresponded to the C-11 enol methine. Attempts at purification proved unsuccessful, the silyl enol ether degrading back to the starting ketone (100b), not surprisingly, on both silica and alumina.

The apparent difficulty in enolising the C-12 ketone in (100b) could be responsible for the lack of reactivity with DBAD or DEAD, and therefore this route was no longer pursued.
3.2 Routes to Target Steroid (86) through Intermediate (110), (Schemes 3 and 5).

The conformational features of steroids is considered to be one of the important factors responsible for their binding to steroid receptors which control hormonal activities\(^2\). \(\Delta^{11}\)-Derivatives of steroids are interesting, because deformation of the C-ring caused by the introduction of the double bond at C11 and C12 positions flattens their A-ring conformation with a 4-en-3-one system which plays a primary role in binding for the receptors. Despite these conformational and physiological features, only a few \(\Delta^{11}\)-derivatives of steroids have been synthesised and no really satisfactory synthetic method has yet been reported, probably due to the intrinsic difficulty of preparing such highly strained molecules.

Pregna-4,11-diene-3,20-dione (\(\Delta^{11}\)-progesterone) was first synthesised by Reichstein \textit{et al.}\(^{208}\) in 1943 and 17\(\alpha\)-methyl-pregna-4,11-diene-3,20-dione was synthesised by Engel \textit{et al.}\(^{208}\) in 1956, both by dehydrotosylation of the corresponding C12\(\alpha\)-tosylates using several different types of base. Dehydrosulfonylation of several C12\(\alpha\)-arylsulfonates of methyl deoxycholate (97) (and C3\(\alpha\)-acetylated derivatives) with potassium tert-butoxide in DMSO\(^{210}\) and reaction of tosylhydrazone of 3\(\beta\)-hydroxy-12-oxocholanic acid methyl ester with phenyl lithium\(^{211}\) have been examined in order to prepare the corresponding \(\Delta^{11}\)-derivatives which were expected to serve as synthetic precursors of the \(\Delta^{11}\)-progestins.

Engel and Popadopoulos\(^{212}\) reported the elimination of bulky C12\(\alpha\)-sulfonates, such as naphthalenesulfonates and tosylates, in the pregnane series by chromatography over aluminium oxide. However, mesylates required elevated temperatures.
Dropwise addition of methane sulfonyl chloride to (99d) in pyridine at 0 °C to gave the mesylate (178) in quantitative yield. The 1H NMR spectrum of (178) showed a singlet at δH 3.12 ppm (SCH₃) and a shift downfield of the CH-12β to δH 5.13 ppm corresponding to the formation of a mesylate. As no starting material was observed by TLC or NMR spectroscopy the material was taken onto the next step without further purification (Scheme 31).

Dehydromesylation of (178) with Bharucha and Schrenk conditions,²¹⁰ potassium tert-butoxide in pyridine, gave 62 % of the olefin intermediate (110, R = Me, R¹ = PhCO) after chromatography. The 1H NMR spectrum of (110, R = Me, R¹ = PhCO) showed characteristic proton signals at δH 5.43 ppm (doublet, J = 10 Hz, CH-12) and 6.11 ppm (double doublet, J = 10 and 3 Hz, CH-11). Switching to a higher boiling aprotic solvent 1,3-dimethyl-3,4,5,6-tetrahydro-2-pyrimidone (DMPU, b.p. 145 - 146 °C / 44 mm) and a weaker base, potassium acetate²¹³, increased the yield to 88 %, most likely due to the less vigorous conditions used for elimination. This is in accordance with work performed within the group.²¹⁴

Tosylates and other bulkier sulfonates are known to undergo elimination in solution by E1 or E2 mechanisms. Generally, only if the base added to abstract a proton (α to the sulfonate) has a marked steric hindrance from the sulphonate or surrounding molecule does E1 elimination take place. In the case of mesylate (178), E2 elimination is more likely as the leaving substituents lie in an anti-coplanar (trans diaxial) system permitting
maximum overlap of orbitals and minimising electronic repulsions giving low transitional state energy (Scheme 32).

Scheme 32

As intermediate (110, $R = \text{Me}$, $R^1 = \text{PhCO}$) was synthesised, functionalisation of the C-ring was attempted firstly through an oxyamination (step i, Scheme 5, Section 2.3).

Sharpless$^{215}$ reported the first vicinal oxyamination of cyclohexene (179) by alkyl imido osmium compounds in the co-ordinating solvent pyridine to give the vicinal amino alcohol (180) in 85% yield.

Scheme 33

One of the most remarkable aspects of this reaction is the preference exhibited by the trioxoalkyl imido reagent for delivery of the nitrogen to one of the olefinic carbons. This reaction pathway would appear to be disfavoured by the steric hindrance in the vicinity of the nitrogen produced by the tertiary alkyl substituents.
A subsequent paper from the same group\textsuperscript{216} reported vicinal oxyamination of cyclohexene (179) and hindered derivatives with the trihydrate of Chloramine-T (181) in the presence of a catalytic amount of osmium tetroxide to produce the vicinal hydroxy \( p \)-toluenesulfonamide derivative (182, 65\%, Scheme 34).

\[ \text{TsNCINa.3H}_2\text{O}^+ \xrightarrow{1\% \text{OsO}_4} \text{t-BuOH, 600°C} \]  
\[ \text{NaCl + } \text{(182)} \]

\[ \text{Scheme 34} \]

\( \Delta^2 \)-Cholestene (183) was oxyaminated using the same procedure, providing two regioisomers (184) and (185), showing that under smaller steric restrictions both molecule faces may be attacked, a bias being towards the \( \alpha \)-face (hindrance from C19 methyl on the \( \beta \)-face).

\[ \text{TsNCINa.3H}_2\text{O}^+ \xrightarrow{1\% \text{OsO}_4} \text{t-BuOH, 600°C} \]  
\[ \{ \text{184, 56\%} \} + \{ \text{185, 31\%} \} \]

\[ \text{Scheme 35} \]

In the case of compound (110, \( R = \text{Me}, R^1 = \text{PhCO} \)), it was envisaged that as C11 and C12 were both sterically confined by their angular methyl groups, if the reaction took place then the nitrogen component may be introduced at either C11 or C12, however preferential approach would be from the less hindered \( \alpha \)-face. Olefin (110, \( R = \text{Me}, R^1 = \text{PhCO} \)) was refluxed with Chloramine-T trihydrate and a catalytic quantity of osmium tetroxide in tert-butyl alcohol for sixteen hours. Work-up provided only unreacted starting material with no incorporation of either an oxygen or nitrogen functionality at either C11 or C12. This result ended the pathway to (86) through (step i, Scheme 5, Section 2.3).
An alternative route through the 11\(\alpha\),12\(\alpha\)-epoxide (111, Scheme 5) was then investigated. Olefinic bonds at most positions in steroidal molecules are attacked by peracids to give \(\alpha\)-epoxides as sole or principle products. From 1950 - 1970's, perbenzoic acid and monoperphthalic acid were the most frequently used epoxidising reagents in the steroidal field. 3-Chloroperoxybenzoic acid (m-chloroperbenzoic acid, mCPBA),\(^{217}\) has proved to be an equally efficient reagent which is commercially available.\(^{218}\)

"Steroid Reactions"\(^{219}\) lists examples of exclusive \(\alpha\)-epoxide formation from \(\Delta^2, \Delta^4, \Delta^6, \Delta^7, \Delta^{8,9}, \Delta^{14}, \Delta^{16}, \Delta^{17,20}\) unsaturated steroids. More steroidal olefin \(\alpha\)-epoxidations with peracids have included \(\Delta^1, \Delta^3, \Delta^4, \Delta^8, \Delta^{11,12}, \Delta^{8,14,224}\) and \(\Delta^{16,226}\).

Treatment of \(\Delta^{11}\)-olefin (110, \(R = \text{Me}, R^1 = \text{PhCO}\)) with mCPBA in chloroform produced 72 % of the \(\alpha\)-epoxide\(^{214}\) (111, \(R = \text{Me}, R^1 = \text{PhCO}\)) with only a trace amount (3 %) of the \(\beta\)-epoxide (186) separated by column chromatography. The observed \(^1\)H NMR spectrum of (111, \(R = \text{Me}, R^1 = \text{PhCO}\)) showed characteristic doublets for the CH-11 and CH-12 protons at \(\delta 2.94\) and 3.14 ppm respectively. The preferred attack by the reagent on the \(\alpha\)-side of the steroid nucleus can be attributed to shielding of the \(\beta\)-side of the molecules by the two angular methyl groups. The general electrophilic addition mechanism of the peracid-olefin reaction is currently believed to involve either an intramolecularly bonded spiro species (187)\(^{226,227}\) or a 1,3-dipolar adduct of a carbonyl oxide (188, Fig. 50).\(^{228}\)

\[
\begin{align*}
\text{(187)} & \quad \text{(188)} \\
\end{align*}
\]

Fig. 50
The reaction probably proceeds through a concerted mechanism in which the alkene (110, R = Me, R' = PhCO) and the electrophilic peroxy oxygen of mCPBA co-ordinates, with concomitant expulsion of the carboxylic acid and release of the epoxide. The transition state for this reaction is usually represented by a complex such as (189, Scheme 36). The geometry of the transition state facilitates loss of the acid by-product (168) and also dictates a syn delivery of oxygen, without changing the geometry of the alkene (Scheme 36).

Scheme 36

Dioxiranes have also been shown to be important and versatile oxidants which may be isolated or generated in situ.\textsuperscript{226,230} The most widely used dioxirane in epoxidations is dimethyldioxirane (191) which can be either isolated and used as a solution in the parent ketone\textsuperscript{231} (Scheme 37) or can be generated in situ\textsuperscript{232} using a biphasic system and a phase transfer catalyst in a buffered system, held at pH 7.5.

In the past, steroidal olefins $\Delta^1$, $\Delta^4$\textsuperscript{233} and $\Delta^5$\textsuperscript{234} have been epoxidised by dimethyldioxirane. Curci\textsuperscript{235} three years after Murray, reported the isolation of methyl(trifluoromethyl)dioxirane, reported to be one thousand times more reactive than dimethyldioxirane (191).\textsuperscript{236}
Work by Yang\textsuperscript{237,238,239} demonstrated efficient \textit{in situ} generation of dioxiranes, in an acetonitrile / water system containing sodium hydrogen carbonate.

Marples \textit{et al}\textsuperscript{40} have shown that using a catalytic amount of 2,2,2-trifluoroacetophenone as the starting ketone, under monophasic conditions,\textsuperscript{241} cholesterol can be converted to its epoxide (60 \% $\alpha$, 40 \% $\beta$) quantitatively. Employing this monophasic method for the epoxidation of (110, \(R = \text{Me}, \ R^1 = \text{PhCO}\)), with acetonitrile / water, the $\alpha$-epoxide (111 \(R = \text{Me}, \ R^1 = \text{PhCO}\)) was exclusively obtained in 96 \% yield with no detection of the $\beta$-epoxide (186) by $^1$H NMR spectroscopy. The electrophilic nature of these epoxidations using dioxiranes has been established and it has been suggested that a \textit{spiro} transition state is involved\textsuperscript{242} and will be discussed in greater detail later (Section 3.3).

Nucleophilic opening of the epoxide ring with a variety of nucleophiles demonstrates the flexibility of these systems. Nucleophilic ring opening can take place under a wide variety of conditions and provides one of the best methods of generating two contiguous stereochemically defined $sp^3$ carbon atoms.\textsuperscript{243} Existing literature precedent describes a range of reaction conditions for the opening of epoxides with nitrogen containing nucleophiles.\textsuperscript{244,245}

Hewett and Savage\textsuperscript{246,247,248} reported the preparation of hydroxy amino functionalised steroids from epoxy-androstanes and -pregnanes using morphilino, piperido and some other nitrogen containing nucleophiles. The authors managed to functionalise the androstane at C6 by nucleophilically opening the corresponding 5$\alpha$,6$\alpha$-epoxide with primary and secondary amines. 3$\beta$-Acetoxy-5$\alpha$,6$\alpha$-epoxyandrostan-17-one (192) gave 3$\beta$,5$\alpha$-
dihydroxy-6β-morpholinoandrostan-17-one (193) after refluxing in aqueous (10 %) morpholine in good yield (Scheme 37).

![Scheme 37](image)

Following step viii (Scheme 5, Section 2.3), α-epoxide (111, R = Me, R' = PhCO) was subjected to a number of different ring opening conditions in order to provide substituted amino alcohol (115, Scheme 5).

The epoxide (111, R = Me, R' = PhCO) was treated with 10 equivalents of benzylamine in the presence of benzylamine hydrochloride and stirred for several days. However, the epoxide moiety remained intact. In order to catalyse the epoxide ring opening with benzylamine as nucleophile, a small amount of lithium perchlorate was added and the reaction repeated. However, no ring opening of the epoxide occurred. Furthermore, heating at 100 °C for one day or refluxing aqueous benzylamine (10 % water) was not successful in opening the epoxide (111 R = Me, R' = PhCO).

There are numerous examples of epoxide ring openings with azide anion nucleophiles applied to saturated steroidal models. Epoxide rings may be opened by rupture of one of the C-O bonds to give "1,2-disubstituted" compounds. Generally, the reaction occurs very readily under acidic catalysis, for protonation of the epoxide ring greatly enhances the normal polarisation of the C-O bonds, permitting nucleophillic attack. In epoxide openings the attacking nucleophile approaches the epoxide from a periplanar direction in order to preserve the maximum overlap of orbitals in the transition state. In steroidal epoxides the effect is to give a trans diaxially substituted product in almost every case. It follows from the principles of
conformational analysis that every steroidal epoxide should yield a unique product or ring opening, with the epoxide oxygen affording an axial hydroxyl group corresponding to the configuration of the epoxide, and the entering nucleophile becoming axially bonded at the other carbon atom.

Campbell et al.\textsuperscript{250} demonstrated the use of azide anion in the ring opening of 2β,3β-epoxy-5α-androstan-17-one (194). \textit{trans-Diaxial} ring opening\textsuperscript{251,252} of (194) by sodium azide gave the 2β-hydroxy-3α-azide (195) as the sole product which was converted to the amine (196) by catalytic hydrogenation (Scheme 39).

![Scheme 39](image)

Reaction of 11α,12α-epoxide (111, R = Me, R' = PhCO) with NaN\textsubscript{3}/DMSO in the presence of concentrated H\textsubscript{2}SO\textsubscript{4}\textsuperscript{253} afforded the 12β-hydroxy-11-oxo-compound (197, 57 %) rather than the expected 11β-azido-12α-hydroxy-compound (198) (Scheme 40). A similar reaction occurred with LiN\textsubscript{3}. The assignment of structure was supported by the NMR spectra which showed peaks at $\delta_H 3.87$ (s, CH-12α) and $\delta_C 213.12$ (C-11 C=O). Furthermore, the positions of the angular methyl groups at $\delta_H 0.61$ (s, C-13-Me) and 1.20 (C-10-Me) are consistent with the 12-hydroxy-11-oxo structure.\textsuperscript{214}

It is possible that the 12β-hydroxy-11-oxo-compound (197) arises from hydrolysis during chromatography of the imine (199) which is formed from the unstable 11β-azido-12α-hydroxy-compound (198). TLC evidence supports the intermediacy of an unstable product. Loss of nitrogen from α-azido-ketones has previously been reported\textsuperscript{154,156,157} and is believed to be sterically driven, in part. The azido group of (198), however, suffers
significant interaction with the angular methyl groups, although loss of nitrogen with formation of the imine (199) would release this (Scheme 40). It seems that this is sufficient to cause the observed decomposition. It would be anticipated that the thermodynamically stable 12β-hydroxy compound would be produced rather than its epimer through an equilibration (cf 153a, 153b, 154a and 154b, page 70). The hydrolysis of the imine (199) supports evidence of loss of C-11 nitrogen functionality in enamine (168) and protected enamines (173b) and (173c) during Luche reduction of the 12-ketone. The inherent instability of the enamine function in the absence of the 12-keto group would leave the C-ring system open to hydrolysis to relieve any steric strain from the angular methyls.

Attempts to prepare the 11β-azido-12α-hydroxy compound (198) using TMSN$_3$/BF$_3$OEt$_2$ in DMF appeared to give the 12α-hydroxy-Δ$^{9,11}$-compound (200, 50 %) presumably by straight Lewis acid-catalysed rearrangement of the epoxide moiety (Scheme 41) without direct reaction with the azide nucleophile.
The structure (200) was supported in particular by the appearance of important signals in the $^1$H NMR spectrum at $\delta_H$ 5.66 (doublet, $J = 6$ Hz, CH-11) and 3.91 (triplet collapsing to a doublet on addition of D$_2$O, $J = 6$ Hz, CH-12(\beta)) and the chemical shifts of the angular methyl groups at 0.57 (s, C13-Me) and 1.12 (s, C10-Me).

No reaction was observed between the 11\alpha,12\alpha-epoxide (111 R = Me, \(R^1 = \text{PhCO}_2\)) and TMSN$_3$ / SmI$_2$ in dichloromethane\textsuperscript{255} which is consistent with work performed within the group\textsuperscript{214}. The epoxide remained intact after seven days at room temperature.

Treatment of terminal epoxides with acetonitrile catalysed by Lewis acids have been reported by Umezawa and co-workers\textsuperscript{256} to produce the corresponding oxazolines, which after acid hydrolysis yield amino alcohols (Scheme 42).
Reaction of epoxide (111, R = Me, R1 = PhCO) with acetonitrile in the presence of five equivalents of aluminium chloride at 0 °C yielded only unidentifiable products inconsistent with the expected oxazoline.

Overall, amine and nitrile nucleophiles were unsuccessful in providing the corresponding target amino-alcohols or oxazolines through ring opening of the epoxide (111, R = Me, R1 = PhCO).

Ring opening of epoxide (111, R = Me, R1 = PhCO) is achieved with NaN₃ in the presence of acid and demonstrates the ring may be opened under more forcing conditions with a smaller linear nucleophile. However the instability of an 11-β azido group (from trans-diaxial ring opening) is subject to the same steric constraints from the angular methyls as that described for azido-ketone (167) as mentioned earlier. The 11β-azido-12α-alcohol (198) then loses nitrogen gas and hydrolys to the equilibrated 12β-hydroxy-11-oxo-compound (197).

Changing the acid to a milder Lewis acid only sets up a faster competing reaction to nucleophilic ring opening resulting in rearrangement of the epoxide (111, R = Me, R1 = PhCO) to the allylic alcohol (200).
3.3 Synthesis of Intermediates (123) and (124), (Scheme 7).

Starting from methyl deoxycholate (98, R = OMe) the following reaction pathways require aromatisation of the A-ring to intermediates (125) and (126) before functionality can be introduced in the C-ring (Scheme 7). Aromatisation of the A-ring would be expected to reduce steric interactions from the angular methyls and therefore permit C ring modification and synthesis of the aromatic target (85, Scheme 8, Section 2.5).

Starting from the already synthesised methyl 3α-benzylloxy-5β-chol-11-en-24-oate\(^{214}\) (110, R = Me, \(R^1 = \text{PhCO}\)), through three steps from methyl deoxycholate (98, R = OMe), deprotection at C3 was achieved through base catalysis, then re-methylation at C24, to yield methyl 3α-hydroxy-5β-chol-11-en-24-oate (110, R = Me, \(R^1 = \text{H}\), 80 %, ROUTE A, Scheme 43). Two-phase chromium oxidation (as discussed earlier in Section 3.1) of (110, R = Me, \(R^1\)
= H) resulted in the formation of the 3-keto $\Delta^{11}$ compound (122) in 72 % yield after recrystallisation from acetone (Scheme 43). The structure (122) was supported in particular by the appearance of important signals in the NMR spectra at $\delta_H$ 5.44 (double doublet, $J = 10$ and 1 Hz, CH-12), 6.17 (double doublet, $J = 10$ and 3 Hz, CH-11) and $\delta_C$ 213.07 ppm (C-3).

As the synthesis of (122) through ROUTE A had five separate steps, one of which involving a capricious base catalysed deprotection, a second route ROUTE B was investigated involving selective oxidation.

Selective oxidation of steroidal C3-\(\alpha\)-hydroxyl in the presence of a C12-\(\alpha\)-hydroxyl has been achieved in the past by a number of methods, silver carbonate on Celite®,257 Oppenauer oxidation258 and Swem oxidation of trimethylsilyl-protected alcohols.259

The Oppenauer reaction has received much attention in preferential oxidation of polyhydroxyl compounds, the superiority of which is exemplified by the one-step oxidation of methyl deoxycholate (98, R = OMe)261 to the corresponding 3-ketone (120, R = Me) in 57 - 63 % yield.

Application of the Oppenauer oxidation to methyl deoxycholate (96) in our case gave methyl 12\(\alpha\)-hydroxy-3-oxo-5\(\beta\)-cholan-24-oate (109) in a slightly higher yield of 65 % after column chromatography. For oxidation of steroidal alcohols the reaction is normally conducted at the boiling point of an inert solvent (toluene) with a large excess of the hydrogen accepting carbonyl compound (cyclohexanone) in the presence of the readily available catalyst aluminium t-butoxide. The mechanism of the oxidation is considered to proceed via the cyclic complex (201) (Scheme 44).
Mesylation (as discussed in Section 2.5) of (109) with methane sulfonyl chloride in pyridine provided the C12α-meslyate\(^\text{264}\) (121, \(R = \text{Me}, 83\%\)) after column chromatography. Elimination of the sulfonate ester (110) occurred smoothly with potassium acetate in DMPU over four hours to yield methyl 3-oxo-5β-chol-11-en-24-oate (122) in 84% yield after chromatography.

ROUTE B only involves three steps and allowed larger quantities of material to be taken through to (122).

The generation of the biologically important 1,4-dien-3-one unit by dehydrogenation of 3-oxo steroids has been an area of intense research for many years (step iv, Scheme 7). The most commonly employed methods are dicyanodichloroquinone (DDQ),\(^\text{265}\) selenium dioxide\(^\text{266,267}\) and bromination-dehydrobromination,\(^\text{268,113,269}\) all having certain synthetic limitations. Dehydrogenation of 3-ketones with two equivalents of DDQ in refluxing benzene gives the Δ\(^1\).\(^4\)-3-ketone in ~ 50% yield\(^\text{270}\) with a lengthy purification as side reactions to Δ\(^4\).\(^6\)-3-ketone and Δ\(^1\).\(^4\).\(^6\)-3-ketone may occur.

Selenium dioxide can be used to introduce Δ\(^1\)-double bonds into 3-ketones in a similar fashion to DDQ, although it is not such a versatile reagent as a constant problem in the use of selenium dioxide is the removal of selenium.
containing contaminants. $\Delta^{14}\text{-3-ketones}$ may be prepared from $5\alpha$- and $5\beta$-
saturated steroids, however in very poor yield.

Bromination of $5\beta$-3-ketones yields the equatorial $4\beta$-bromo compound,
which when further brominated under thermodynamic or kinetic conditions,
yields the $2\beta,4\beta$-dibromo compound containing a little of the $2,2$-dibromo
isomer. Dehalogenation introduced by Joly,$^{268}$ involving the use of an
excess of lithium carbonate in DMF, gives a clean method to $\Delta^{14}\text{-3-ketones}$
from $2,4$-dibromo-3-ketones. The excess lithium carbonate presumably
prevents the build up hydrogen bromide. As all three procedures above
involve several steps coupled with difficult work ups, an alternative multi-
step procedure was required to take an adequate quantity of material
through to the trieneone (123, $R = \text{Me}$, Scheme 7, Section 2.5).

Barton and co-workers$^{271}$ showed that the dehydrogenation of steroidal 3-
ketones can be accomplished in high yield using benzeneselenenic
anhydride generated in situ by efficient oxygen atom transfer from
iodoxybenzene to catalytic amounts of diphenyl diselenide. An
experimentally convenient and economical development of this catalytic
cycle was the use of $\text{meta}$-iodoxybenzoic acid which both avoids
chromatography and allows the recovery of $\text{meta}$-iodobenzoic acid and
diphenyl diselenide.

$\text{meta}$-iodoxybenzoic acid, diphenyl diselenide, and (122, $R = \text{Me}$) were
refluxed in toluene for thirty-six hours. After chromatography, methyl 3-oxo-
$5\beta$-chola-$1,4,11$-trien-24-oate (123, $R = \text{Me}$, Scheme 7) was isolated in 67 %
yield,$^{272,273}$ which gave the observed characteristic peaks in the $^1\text{H}$ NMR
spectrum; $\delta_{\text{H}}$ 5.54 - 5.57 (double doublet, $J = 10$ and 2 Hz, C12-H), 6.11 -
6.12 (doublet, $J = 1$ Hz, C4-H), 6.26 (double doublet, $J = 10$ and 3 Hz, C11-
H), 6.27 (double doublet, $J = 10$ and 1Hz, C2-H), 7.12 - 7.15 (doublet, $J = 10$
Hz, C1-H).

This catalytic method for dehydrogenation requires long reaction times and
is only useful for taking small quantities of (122, $R = \text{Me}$) through to trienone
108
(123, R = Me), typically below fifty milligrams. Using larger quantities of starting material caused the yields of (123, R = Me) to drop dramatically. Earlier work by Barton and co-workers\textsuperscript{274} reported the dehydrogenation of 3-ketones using stoichiometric quantities of benzeneselenenic anhydride (BSA) in hot / refluxing chlorobenzene, giving good yields of A-ring dienones in less than three hours. Application of this method, with addition of some meta-iodoxybenzoic acid (to convert diphenyl diselenide to BSA) to 220 mg (122, R = Me) gave three products after an oxidative work-up with hydrogen peroxide\textsuperscript{275} and chromatography (Scheme 45). Hydrogen peroxide was used in the work up to convert any \(\alpha\)-phenylselenyl ketones formed, to their respective selenoxides, which are known to eliminate at room temperature to their respective enones.\textsuperscript{276}

![Scheme 45](image)

As can be seen from the ratios of products (202), (203) and (123, R = Me), the 5\(\beta\) series mainly gives the \(\Delta^{1,4}\)-3-ketone predominantly through the \(\Delta^{4}\)-3-ketone, as expected\textsuperscript{277} when compared with DDQ.

The mechanism of unsaturation of (122, R = Me) by BSA and the \textit{in situ} BSA methods involve electrophilic attack upon the kinetically favoured enol by benzeneselenenic anhydride. The resulting 2-phenylseleno steroid could then decompose either by a loss of a proton at C1 concerted with a detachment of PhSe(O)\textsuperscript{-} (PATH A, Scheme 46) to the \(\Delta^{1}\)-compound or, by 1,4-elimination of the PhSe(O)\textsuperscript{-} group with the 5\(\beta\) proton to give the \(\Delta^{4}\)-compound (PATH B, Scheme 46). In either case a second reaction takes
place to introduce the second double bond in the other position ($\Delta^4$ or $\Delta^1$) resulting in the formation of trienone (123, R = Me).

One consequence of this reaction, reducing overall yield of (123, R = Me), is the 2-phenylseleno steroid may perform a Pummerer type reaction, with the loss of the $\alpha$-proton leading to an $\alpha$-diketone. The A-nor steroid may then arise via benzilic acid rearrangement of the corresponding 1,2-dicarbonyl species.276 These are derived as outlined in Scheme 47 for which ample precedence exists.279,280

Epoxidation of trienone (123, R = Me) under standard mCPBA conditions217 in chloroform yielded the 11$\alpha$,12$\alpha$-epoxide272,273 (124, R = Me) in 82 % yield (Table 10), the structure of which was supported by the IR spectrum ($v_{\text{max}}$ 1664 cm$^{-1}$, $\alpha\alpha'$, $\beta\beta'$- unsaturated ketone) and the $^1$H NMR spectrum; $\delta_H$ 3.18 (doublet, $J = 4$ Hz, C12-H), 3.23 (doublet, $J = 4$ Hz, C11-H), 6.10 (broad singlet, C4-H), 6.33 (double doublet, $J = 10$ and 2 Hz, C2-H) and 7.25
(doublet, \( J = 10 \text{ Hz}, \ C1-H \)) ppm. The \( ^1\text{H} \) NMR spectrum also revealed an isomeric ratio of 5:1 \((\alpha:\beta)\) respectively, after integration of epoxide methines \((\delta^H 3.05 (\beta) \text{ and } 3.18 (\alpha) \text{ ppm})\).

Employing the already mentioned \textit{in situ} catalytic epoxidation method with 2,2,2-trifluoroacetophenone as the starting ketone, trienone (123, \( R = \text{Me} \)) was epoxidised in 96 \% yield with a higher ratio of \( \alpha \)-epoxide \((7:1 (\alpha : \beta) \text{ based upon } ^1\text{H} \text{ NMR data})\). Precursor compounds (122, \( R = \text{Me} \)) and (202) of trienone (123, \( R = \text{Me} \)) were also epoxidised by the \textit{in situ} method at \( \Delta^{11} \) to give predominantly the \( \alpha \)-epoxide in excellent yields (Table 10), showing the high selectivity of this method.

The \textit{in situ} method has advantages over \textit{m}-CPBA for epoxidation of \( \Delta^{11} \) olefins:

- the method is catalytic and relatively inexpensive;
- reaction work-up is uncomplicated giving high yields;
- and selectivity for the \( \alpha \)-face is increased due to steric factors around the C-ring.

Increasing the size of epoxidising agent from \textit{m}CPBA to the 2,2,2-trifluoroacetophenone derived dioxirane results in almost exclusive attack from the \( \alpha \)-face.

From the work by Curci and co-workers\textsuperscript{235} it is known that the oxidising ability of the dioxirane is improved by the introduction of the powerful electron withdrawing trifluoromethyl group, \( \alpha \) to the dioxirane ring. The trifluoromethyl group can be expected to influence the stability of the small ring in several ways. For one by drawing electron density from the negative oxygen pole towards the \( \alpha \)-carbon, it would discourage formation of dipole isomers (206), then any reaction that is initiated \textit{via} carbon-oxygen bond rupture of (207) \textit{e.g.} dimerisation to the 1,2,4,5-tetraoxane ('ketone superoxide') would be disfavoured.\textsuperscript{230}
Table 10 - Epoxidations Using m-CPBA and In Situ Dioxirane Method.
The effect on the reactivity may perhaps be explained by an increase in electrophilicity of the dioxirane (208). The electron deficiency at the carbonyl atom may assist oxygen transfer of the dioxirane by moving the equilibrium towards ketone formation.

The mechanism of epoxidation by a dioxirane can be viewed in terms of the transition state of the two extremes for the electrophilic oxygen atom transfer; planar or spiro (Fig. 52). Epoxidation by peroxyacids is generally viewed as being a cyclic planar concerted process ("butterfly" transition state)\textsuperscript{225}, where generally an increasing number of alkyl substituents in the alkene increases the reactivity.

![Planar and Spiro Transition States](image)

Fig. 52

The now generally recognised mechanism involves the spiro transition state proposed by Baumstark, based upon the observation that cis-olefins were more reactive than the corresponding trans-olefins for epoxidation using dimethyl dioxirane\textsuperscript{283}. This assumption has been supported by Shi et al\textsuperscript{284} who also proposed a spiro model for the epoxidation of (R,R) and (S,S) stilbene by a fructose derived ketone.

The in situ method has proven to be a highly specific, reliable method for the epoxidation of $\Delta^{11}$ in the deoxycholic acid series.

The target intermediates (123, R = Me) and (124, R = Me) have been successfully prepared from methyl deoxycholate (98, R = OMe) in four and five steps respectively.
### 3.4 Synthesis of Intermediates (133) and (134), (Scheme 9).

<table>
<thead>
<tr>
<th>Starting material</th>
<th>Reaction Conditions</th>
<th>Reaction Time (h)</th>
<th>$\Delta^{1,4}$-Dien-3,12-dione (133, R = Me)</th>
<th>$\Delta^{1,4,9(11)}$-Trien-3,12-dione (134, R = Me)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td>2 equiv. SeO$_2$, t-BuOH, reflux</td>
<td>24</td>
<td>32%</td>
<td>-</td>
</tr>
<tr>
<td><img src="image2.png" alt="Image" /></td>
<td>2 equiv. DDQ, benzene, reflux</td>
<td>18</td>
<td>40%</td>
<td>-</td>
</tr>
<tr>
<td><img src="image3.png" alt="Image" /></td>
<td>(PhSe)$_2$, m-iodoxybenzoic acid, toluene, reflux</td>
<td>72</td>
<td>-</td>
<td>64%$^{271}$</td>
</tr>
<tr>
<td><img src="image4.png" alt="Image" /></td>
<td>(PhSe)$_2$, m-iodoxybenzoic acid, toluene, reflux</td>
<td>36</td>
<td>76%</td>
<td>3%</td>
</tr>
<tr>
<td><img src="image5.png" alt="Image" /></td>
<td>(PhSe)$_2$, m-iodoxybenzoic acid, toluene, reflux</td>
<td>72</td>
<td>5%</td>
<td>62%</td>
</tr>
<tr>
<td><img src="image6.png" alt="Image" /></td>
<td>3 equivs BSA, chlorobenzene reflux</td>
<td>4</td>
<td>~ 2%</td>
<td>60%</td>
</tr>
</tbody>
</table>

**Table 11 - Summary of Dehydrogenations of (132, R = Me).**

Analogous to (122, R = Me), dehydrogenation of (132, Scheme 9, Section 2.5) was performed to introduce the 1,4-dien-3-one system as proposed in Chapter 2 (Scheme 9, Section 2.5).
Tsuda et al. previously synthesised $\Delta^{1,4}$-dien-3,12-dione (133, $R = \text{Me}$) and $\Delta^{1,4,9(11)}$-triene-3,12-dione (134, $R = \text{Me}$) in very poor yields through a number of different dehydrogenation techniques (Scheme 48). The most successful method of synthesising (134, $R = \text{Me}$) was to form the $\Delta^{8,11}$ 3,12-dione (209) through Fieser's procedure from deoxycholic acid (97) and then to dehydrogenate with selenium dioxide in tert-amyl alcohol.

![Scheme 48](image)

Oxidation of methyl deoxycholate (98, $R = \text{OMe}$) to 3,12-dioxo-5β-cholan-24-oate (132, $R = \text{Me}$) proceeded smoothly with a two-phase chromium mediated oxidation (as described in Section 3.1) to give the product as white prisms (60%) from methanol.

Attempts at synthesising dienedione (133, $R = \text{Me}$) from the diketone (134, $R = \text{Me}$) with selenium dioxide in refluxing t-butanol proved successful (32%), however purification was lengthy (Table 11). An increase in reaction times of up to three days gave no further unsaturation at $\Delta^{8,11}$ to yield intermediate (134, $R = \text{Me}$) however, decreased yields of (133, $R = \text{Me}$) to less than 20%.

Refluxing three equivalents of DDQ overnight with (132, $R = \text{Me}$) in benzene resulted in a complicated mixture of products which when purified gave the
dienedione (133, R = Me) in 40 % yield with no observed formation of trienedione intermediate (134, R = Me, Table 11).

Barton and Ley\textsuperscript{271} applied their catalytic dehydrogenation method (Section 3.3), using \textit{meta}-iodobenzoic acid with diphenyl diselenide, to methyl deoxycholate (96). This catalytic dehydrogenation of (98, R = OMe) furnished a spectacular example of consecutive reactions, as the trienedione (134, R = Me) was obtained in 64 % yield after seventy-one hours. Allowing for enolisation, thirteen consecutive steps are involved with an average yield of approximately 97 % for each.

We found that complex mixtures of products were formed when following the same procedure to catalytically dehydrogenate methyl deoxycholate (98, R = OMe). Repeating the reaction a number of times only proved the unreliability of this reaction. Direct application of this catalytic method to the 3,12-diketone (132, R = Me) and therefore reducing the number of consecutive steps required, gave different ratios of products depending on reaction time (Table 11). If the reaction was stopped after eighteen hours, the major product was methyl 3,12-dioxo-5\beta-chol-4-en-24-oate (210, 70 %) with small amounts of methyl 3,12-dioxo-5\beta-chol-1-en-24-oate (211, 3 %) present. However, longer times of 36 h and 72 h gave major products of methyl 3,12-dioxo-5\beta-chol-1,4-dien-24-oate (133, R = Me, 76 %) and methyl 3,12-dioxo-5\beta-chol-4-en-24-oate (134, R = Me, 62 %) respectively.

Using three equivalents of BSA in refluxing anhydrous chlorobenzene gave the triene-dione (134, R = Me, 60 %) after chromatography, consistent with the catalytic BSA method (Table 11).

In conclusion, we feel that benzeneseleninic anhydride (formed \textit{in situ} or otherwise) is an attractive alternative, particularly in comparison to selenium dioxide or DDQ, for the dehydrogenation of steroidal 3- and 11 or 12- ketones. The yields of (133, R = Me) and (134, R = Me) may be varied by changing the reaction time. The major by-product of the reaction, diphenyl diselenide,
can be easily separated and re-oxidised to the anhydride using an oxygen transfer from \textit{m}-iodoxybenzoic acid.

3.5 A-Ring Aromatisation of Intermediates (123), (124), (133) and (134) (Schemes 7 and 9).

The principal approach to aromatisation in organic chemistry has been dehydrogenation. While extremely important in structure work, it can suffer from a nearly total lack of selectivity, the end product usually being a completely aromatic substance, which is often in poor yield.

In the steroidal field, there exists an important incentive to the solution of the problem, namely the synthesis of the oestrogen hormones (possessing one aromatic ring) from precursors, which contain four non-aromatic rings and where simple aromatisation was blocked by the presence of the two angular methyl groups. In order to comply with the original pathways (Schemes 7 and 9) expulsion of the angular methyl group was required and not rearrangement.

Aromatisation of steroidal $\Delta^{14}$-dien-3-ones is well known and includes (Lewis) acid catalysed dienone-phenol rearrangements in which the C-19 is retained and demethylations (reductive aromatisation) involving reaction with lithium and biphenyl, activated zinc or via a pyrolytic elimination.\textsuperscript{287}

Pyrolytic methods have been successful, although yields are invariably low (Table 12). At temperatures above 500 °C the C10-C19 bond breaks homolytically to give a pair of free radicals. With very short reaction times and in the presence of a hydrogen donor (mineral oil) rupture may occur.
Mineral oil, 600 °C, 1-2 ml/min passed through hot glass packed column

**Scheme 49**

<table>
<thead>
<tr>
<th>Compound</th>
<th>$R^1$</th>
<th>$R$</th>
<th>Other function</th>
<th>Yield of (213)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(212a)$^{288}$</td>
<td>Ac</td>
<td>H</td>
<td>$\Delta^{16}$</td>
<td>~ 25 %</td>
</tr>
<tr>
<td>(212b)$^{289}$</td>
<td>OH</td>
<td>H</td>
<td>2-Me</td>
<td>16 %</td>
</tr>
<tr>
<td>(212c)$^{290}$</td>
<td>=O</td>
<td>H</td>
<td>$\Delta^6$</td>
<td>40 – 52 %</td>
</tr>
<tr>
<td>(212d)$^{291}$</td>
<td>=CHCH$_2$OAc</td>
<td>OH</td>
<td>-</td>
<td>15 %</td>
</tr>
<tr>
<td>(212e)$^{292}$</td>
<td>OH</td>
<td>-</td>
<td>-</td>
<td>17 %</td>
</tr>
</tbody>
</table>

**Table 12 - Pyrolytic Aromatisation with Expulsion of Angular Methyl Group.**

Homolysis of the allylic C10-C19 bond must be assisted by developing overlap in the transition state between the C10 orbital containing the odd electron and the $\pi$-orbital system of the dienone. The intermediate product would be the resonance-stabilised phenoxy radical (214), which can accept a hydrogen atom from a suitable donor to give the phenol (Scheme 50).

**Scheme 50 - Resonance Stabilised Phenoxy Radical (214).**
Due to the normally low yields of aromatised products produced and difficult work-up normally obtaining ‘tarry’ products, these pyrolytic methods were not attempted.

Reductive aromatisation of 1,4-dienones in the past, have also been achieved with expulsion of the C19 angular methyl group, by heating with lithium metal and biphenyl in tetrahydrofuran. An excess of the radical dianion from lithium metal and biphenyl in boiling THF solution effects aromatization of the A-ring with expulsion of the angular methyl group. Biphenyl acts as an electron carrier, first forming a lithio derivative (215) of the mesomeric radical anion. The dianion expels a methyl carbanion, probably assisted by one of the lithium cations to form the aromatised A-ring (216) (Scheme 51).

![Scheme 51](image)

Addition of an ‘acidic’ hydrocarbon (diphenylmethane or methylnaphthalene) to trap any methyl lithium formed as methane, minimises the reaction of methyl lithium with unreacted dieneone and therefore increases yields.

![Scheme 52](image)

Baran\textsuperscript{203} incorporated this style of reductive aromatisation in the preparation of 11\textbeta-hydroxyestrone (217, 72 %, Scheme 52), for investigation of
biological interest. However, the reaction involving activated zinc in pyridine containing a trace of water is considered to be the most efficient and was therefore attempted.

Expulsion of the angular methyl group from C10 has most importantly been achieved by treatment of 1,4-dienones with zinc in a non-acidic solvent. The reactions with zinc are thought to involve a single electron addition leading via the radical anion (219) and either a 9,10-seco-C-9 radical (220) or loss of a methyl radical (221) (Scheme 53) to the para-phenol (222) or the 3-hydroxy compound (224) respectively. However, it has been suggested that in certain cases zinc may act as a Lewis acid.284

![Scheme 53](image)

A number of Δ1,4-3-ones have previously been examined (Table 13) and the substituent effects are consistent with a mechanism involving the radical anion (219).
<table>
<thead>
<tr>
<th>C17 Function</th>
<th>C-Ring Functionality</th>
<th>Solvent</th>
<th>CH₃-19 Expulsion</th>
<th>Diene-Phenol Rearrangement</th>
<th>C₁₀-C₆ Scission to Seco Cmpd.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ⁹,¹¹-12-one</td>
<td>Pyridine</td>
<td>70%²⁸⁵</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-OAc</td>
<td>Δ⁶,¹¹-12-one</td>
<td>EtOH</td>
<td>90%¹¹⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>=O</td>
<td>11β-OH</td>
<td>Pyridine</td>
<td></td>
<td>80%²⁸⁵</td>
<td></td>
</tr>
<tr>
<td>=O</td>
<td>Δ⁸,⁻¹⁻⁻⁻-11β-OH</td>
<td>DMF</td>
<td>20%²⁹⁵</td>
<td></td>
<td></td>
</tr>
<tr>
<td>=O</td>
<td>Δ⁸,⁻¹⁻⁻⁻-11-one</td>
<td>Ethylene Glycol</td>
<td>Low yield²⁹⁶</td>
<td></td>
<td>Low yield</td>
</tr>
<tr>
<td>=O</td>
<td>11-one</td>
<td>Pyridine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>=O</td>
<td>Δ⁹,¹¹</td>
<td>Pyridine</td>
<td>75%²⁹⁶</td>
<td></td>
<td></td>
</tr>
<tr>
<td>=O</td>
<td>Δ⁹,¹¹</td>
<td>Pyridine</td>
<td>70%²⁹⁶</td>
<td></td>
<td></td>
</tr>
<tr>
<td>=O</td>
<td>Δ⁹,¹¹</td>
<td>Pyridine</td>
<td>35%²⁹⁶</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 13 - Literature Aromatisations of 1,4-Dien-3-ones using Activated Zinc.**

In aprotic, neutral, or basic solvents, two reactions are open to the radical anion (219). Fragmentation (10,19-cleavage) (221), with expulsion of the...
angular methyl group as a free radical would give the phenoxide ion. The trace of water that is required for reaction may function at this stage in providing a hydrogen atom so that the methyl group can emerge as a molecule of methane.

Rupture of the C9-C10 bond must be an intrinsically more favourable process, for it would give a secondary carbon free radical (220). Recyclisation by attack of the C9 radical on the ortho-position of the phenoxide ring (C4) would give a mesomeric radical-anion which needs to lose a hydrogen radical to the reaction medium to form a stable phenoxide ion, which then picks up a hydrogen atom from the trace water present to give (222).

C-ring functionality plays a very important part in determining which pathway the radical reaction takes place. The reactions of $\Delta^{9,11}$, $\Delta^{9,11}$-12-one and 11-oxo-1,4-dienone compounds are entirely consistent with the radical-anion concept. The effect of $\Delta^{9,11}$ is to favour angular methyl expulsion by providing allylic activation of the C10-C19 bond. An 11-oxo functionality stabilises the C9 radical as a resonance hybrid, which should have a lifetime sufficient to extract a hydrogen atom from the trace amounts of water present forming the C9-C10 seco-compound (224).

We examined the reaction of $\Delta^{1,4,9(11)}$-triene-3,12-dione (225, Table 14) with zinc / pyridine / water, as previously reported, which gave a high yield (90%) of the A-ring aromatic-$\Delta^{9(11)}$-12-ketone (229) whereas the $\Delta^{1,4}$-dieno-3,12-dione (226, Table 14) gave the para-phenol (230, 86%), the product of the dienone-phenol rearrangement (Table 14), instead of expulsion of a methyl radical as suggested in the original pathway (Scheme 9).

Treatment of (227) with zinc under the usual conditions gave a complex mixture (Table 14). Although it was apparent from the $^1$H NMR spectrum of the crude product that aromatisation had occurred, it was not possible to satisfactorily purify the components of the mixture.

122
<table>
<thead>
<tr>
<th>Starting 1,4-dien-3-one</th>
<th>Reaction conditions</th>
<th>Product</th>
<th>Yield</th>
<th>Reaction Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>(225)</td>
<td>Zn / Pyridine / H₂O Reflux 18 h</td>
<td>(229)</td>
<td>90 %</td>
<td>10-Methyl Expulsion</td>
</tr>
<tr>
<td>(226)</td>
<td>Zn / Pyridine / H₂O Reflux 18 h</td>
<td>(230)</td>
<td>86 %</td>
<td>Dienone-Phenol Rearrangement</td>
</tr>
<tr>
<td>(227)</td>
<td>Zn / Pyridine / H₂O Reflux 18 h</td>
<td>Intractable mixture</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(228)</td>
<td>Zn / Pyridine / H₂O Reflux 18 h</td>
<td>(231)</td>
<td>97 %</td>
<td>9,10-Fission</td>
</tr>
</tbody>
</table>

Table 14 - Aromatisations performed with Activated Zinc in Pyridine / Water.

Similar treatment of the 11α,12α-epoxide (228) gave the 9,10-seco-Δ⁶,¹¹-12-ketone (231) which gave the observed characteristic peaks in the IR spectrum ($v_{max}$ 3388 cm⁻¹, OH; 1682 cm⁻¹, α,β-unsaturated ketone) and in the $^1$H NMR spectrum; δ₉ 2.21 (aromatic methyl), 5.86 (double doublet, $J = 3$ and 10 Hz, C11-H), 6.58 (double doublet, $J = 3$ and 8 Hz, C2-H), 6.62 (doublet, $J = 3$ Hz, C4-H), 6.66 (double doublet, $J = 2$ and 10 Hz, C9-H) and 6.98 (doublet, $J = 8$ Hz, C1-H) ppm.

All of the results are best explained by the intermediacy of the radical anion (219, Scheme 53). As reported before, loss of the methyl is facilitated by a Δ⁸,¹¹ double bond. The products from (226), (227) and (228) all arise from the C-9 radical (220, Scheme 53), and in the case of the 11α, 12α-epoxide (228), the reaction is particularly efficient owing to the trapping of the C-9 radical (233) by the epoxide followed by expulsion of a hydrogen radical by allylically activated β-cleavage of the oxyl radical (234) (Scheme 54).
The failure of the $\Delta^{1,4,11}$-trien-3-one (227) to give a single product was expected since the reaction would proceed through the C-9 allylic radical (236, Scheme 55).

It is unlikely that Lewis-acid catalysis is involved in the epoxide reaction since treatment of the 11$\alpha$,12$\alpha$-epoxide (228) with BF$_3$.Et$_2$O in diethyl ether or SnCl$_4$ in dichloromethane gave complex mixtures.

Also, an alternative reaction involving preliminary reduction of the epoxide seems unlikely since the saturated 11$\alpha$,12$\alpha$-epoxide (205, Table 10)$^{268}$ is completely unreactive with zinc / pyridine / water. All four of these results support the evidence of a radical anion mechanism.
Dehydrogenation using BSA or its catalytic *in situ* method has proven to be very effective in producing the three 1,4-dien-3-ones \( \Delta^{11} \) (227), 12-ketone (226) and \( \Delta^{9,11} \)-12-ketone (225).

*In situ* dioxirane epoxidation has proven to be highly effective at the \( \Delta^{11} \) position, especially in producing the required \( 11\alpha,12\alpha \)-epoxy-1,4-dien-3-one (228).

Successful aromatisation occurred in 1,4,\( \Delta^{9,(11)} \)-trien-3,12-dione (225) to give the de-methylated (229) and 1,4-dien-3,12-dione (226) to give the rearranged (230).

However, aromatisation of 1,4-dien-3-ones, \( \Delta^{11} \) (227) and \( 11\alpha,12\alpha \)-epoxide (228), did not yield the expected estrogenic intermediates (125) and (126) (Scheme 7) only giving an inseparable mixture and the 9,10-seco-\( \Delta^{9,11} \)-12-ketone (231) respectively. These two results effectively made Scheme 8 (Section 2.5) redundant and therefore no longer pursued.
3.6 Routes to (85) through 12-Ketone (135) or α,β-Unsaturated Ketone (136) (Scheme 10).

As routes through compound (135, Scheme 9, Section 2.6) were now unavailable from (133), the product of dienone-phenol rearrangement (230, Table 14) was used instead for the rest of the Scheme 10 (Section 2.6).

Acetylation of the (230, Table 14, Section 3.5) proceeded smoothly with acetic anhydride in pyridine overnight at room temperature to give the C1-OH protected estrogen (237, 98%). Following step i (Scheme 10, Section 2.6) protection of C1-OH was necessary for attempting the electrophillic amination at C11 using LDA (as discussed earlier in Section 3.1) as the lithium may promote problems at the phenol, hindering attack from the aminating species.

As the steric hindrance of the C-ring has now been slightly relieved due to the absence of the C19 methyl, the insertion of a nitrogen species at C11 would appear to be more favourable. However, no reaction took place when using either DBAD or DEAD with LDA, possibly due to hindrance from the acetyl group. Attempted amination using the unprotected derivative (230) also proved unsuccessful with the same conditions.
In order to comply with Scheme 10 (Section 2.6), reduction of A-ring aromatic-$\Delta^{6(11)}$-12-ketone (229) was attempted using Luche conditions (as described earlier in Section 3.1). A change in starting material (229) was observed by TLC, however, only starting material was isolated after work-up. A colour change to bright green was observed on addition of sodium borohydride which intensified under a UV lamp. This result suggests a possible resonance stabilised active chromophore in solution due to enolisation at the C-12 ketone, however, this was not further investigated.

A reduction was then attempted upon (229) using Adams' catalyst with hydrogen in acetic acid / ethanol (as discussed earlier for protected enamines 173b and 173c in Section 3.1). Upon purification only 2 mg of pure compound was isolated from a complex mixture, deduced as methyl 3-hydroxy-12-oxo-19-nor-chola-1,3,5(10)-trien-24-oate (239) by a 1,2 adsorption of hydrogen rather than the expected 1,4.

![Chemical structures](image)

**Scheme 57**

The $^1$H NMR spectrum of (239) showed a disappearance of the C-11 methine associated with the unsaturated C-ring of (136, $R = Me$) at $\delta_H$ 6.6 ppm. This result can be backed by Tsuda's work, in which compound (136, $R = Me$) is hydrogenated without selectivity with palladium upon charcoal at both at $\Delta^{6,11}(1,2$ adsorption) and 12-ketone (1,4 adsorption) with removal of the C-24 methyl ester.
Protection of (229) at C-3 hydroxyl with dimethyl sulfate in the presence of K$_2$CO$_3$ proved unsuccessful. However, protection with pyridine / acetic anhydride gave methyl 3-acetoxy-12-oxo-19-nor-chola-1,3,5(10),9(11)-tetraen-24-oate (240) in 98% yield.

Scheme 58

As only a very small amount of (239) was available and due to time constraints of the project, this pathway was stopped at this point.

Future work on this project may involve bromination and azide displacement upon methyl 3-hydroxy-12-oxo-19-nor-chol-1,3,5(10)-trien-24-oate (239) and / or methyl 1-acetoxy-4-methyl-12-oxo-5β-chola-1,3,5(10)-trien-24-oate (237) leading to target estrogenic target compound (86, Fig. 43).
Chapter 4.
4.1 Biological Results.

The detailed understanding of the growth of HIV has enabled chemists to produce compounds targeted at specific sites in the HIV replicative cycle. HIV infects human T lymphocytes, monocytes / macrophages and nerve cells both in AIDS patients and *in vitro*. Compounds have been shown to exhibit differences in antiviral activity and cytotoxicity depending on the cell type studied. It is therefore necessary to evaluate prospective anti-HIV drugs in multiple cell lines of different origin.

Assay systems using specific cell / virus combinations are now available for the assessment of compounds with various mechanisms of anti-HIV action.

In the study of structure-activity relationships of chemically synthesised compounds, small differences in structure can markedly affect the efficacy of any compound.

Compounds (168), (173a), (173b) and (173c) were tested using several different methods in C8166 cells infected with HIV-1(111B) at MRC Collaborative Centre, Mill Hill, London (Fig. 53).

(168) \( R = H \)
(173a) \( R = C_2H_5OCO- \)
(173b) \( R = C_6H_5CH_2OCO- \)
(173c) \( R = (CH_3)_3CCO- \)

Fig. 53

Briefly the methods involved used five fold dilutions of compounds (168), (173a), (173b) and (173c) are made in a 96 well plate (for each compound) and mixed with C8166 cells prior to the addition of virus HIV-1MN (a laboratory adapted strain) at a low multiplicity of infection. The cultures are
incubated at 37 °C. After 5 days of incubation, the inhibition of infection was measured by several criteria as follows.

The cultures were examined microscopically for evidence of syncitia and cytotoxicity. This is a visual technique to look for large multinuclear cells from which 'bud' viral cells (replication). However this method is not totally suited for testing compounds inhibiting at the late stage of virus replication such as protease inhibitors.

Measurement of the cells viability of HIV-infected and uninfected cells by the soluble Formazan assay quantitated the cytopathic effect in infected cells and cytotoxic effect in uninfected culture. The Formazan assay is based upon the metabolic reduction of XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino)carbonyl]-2H-tetrazoliumhydroxide) to a brown soluble product (XTT-Formazan) in surviving cells, the concentration of which may be measured by UV spectroscopy ($\lambda_{test} = 450$ nm and $\lambda_{reference} = 650$ nm). The estimation of the yield of infectious virus is the only reliable method for the assessment of compounds affecting virus maturation and infectivity such as protease inhibitors.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conc. (µM)</th>
<th>Syncytia (+/-)</th>
<th>Progeny Virus % of Control</th>
<th>Estimated Cell Growth % of Control</th>
<th>$EC_{50}$ (µg mL$^{-1}$)</th>
<th>$TC_{50}$ (µg mL$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(168)</td>
<td>50</td>
<td>+/-</td>
<td>&lt;1</td>
<td>41</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>+/-</td>
<td>75</td>
<td>42</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>100</td>
<td>32</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>(173b)</td>
<td>1000</td>
<td>+/-</td>
<td>25</td>
<td>60</td>
<td>200</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>+/-</td>
<td>50</td>
<td>50</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>+</td>
<td>100</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(173c)</td>
<td>100</td>
<td>-</td>
<td></td>
<td>50</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>+/-</td>
<td>&lt;1</td>
<td>50</td>
<td>57</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+/-</td>
<td>75</td>
<td>33</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>+</td>
<td>100</td>
<td>32</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>(173a)</td>
<td>200</td>
<td>+/-</td>
<td>1-5</td>
<td>49</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>+/-</td>
<td>100</td>
<td>50</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>+</td>
<td>100</td>
<td>28</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>AZT (70)</td>
<td>10</td>
<td>-</td>
<td></td>
<td>100</td>
<td>0.016</td>
<td>&gt;1000</td>
</tr>
<tr>
<td></td>
<td>0.016</td>
<td>+</td>
<td>50</td>
<td>44</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Sequinavir (26)</td>
<td>20</td>
<td>-</td>
<td>100</td>
<td>51</td>
<td>0.001</td>
<td>20</td>
</tr>
</tbody>
</table>
TC$_{50}$ ($\mu$g mL$^{-1}$) represents the concentration which reduces Ag glycoprotein 120 by 50% in uninfected cell cultures (the higher the better toxicity rating). EC$_{50}$ ($\mu$g mL$^{-1}$) represents the concentration of drug which reduces cell growth by 50% in infected cell cultures (the lower the better HIV-1 Protease inhibitory action). Table 15 shows parent enamine (168) with an efficacy of EC$_{50} = 20$ $\mu$g mL$^{-1}$ and low toxicity rating TC$_{50} = 50$ $\mu$g mL$^{-1}$ comparable to that of the Roche protease inhibitor Sequinavir (26, Fig. 15, Section 1.8.1).

Substitution of a proton upon the enamine to the carbamate Cbz (173b) resulted in a twenty fold decrease in toxicity, however, with a ten fold decrease in protease activity. Switching the carbamate group from Cbz (173b) to ethoxycarbonyl (173c) increased the efficacy of the inhibitor, but also increased it's toxic effects in killing non-infected cells. The pivaloyl derivative (173c) proved to be the most active. If compared to Roche's HIV-1 protease inhibitor Sequinavir (26), the toxicity rating is comparable. However (173c) is not as active. Energy minimisations of (168), (173a), (173b) and (173c) were performed upon CAChe ver. 4.0 Molecular Modelling package and are shown in Figs 54 - 57.
Fig. 54 - Energy Minimisation of (168).

Fig. 55 - Energy Minimisation of (173b).
Fig. 56 - Energy Minimisation of (173a).

Fig. 57 - Energy Minimisation of (173c).
The main observation to be taken from the energy minimisations (Figs 54 - 57) is that, in all four cases the steroid conformation is very similar, the only change is the shape at the R group. It has been shown by Rosenberg et al and Thaisvirongs that increasing a renin inhibitor's hydrophilicity can lead to an improvement in efficacy, possible due to their higher solubility within the body which then results in superior absorption. Similar effects may be observed in Table 15.

Increasing the lipophilicity of the steroid from parent enamine (168) to pivaloyl derivative (173c) shows a general increase in efficacy of inhibitor. Cbz derivative (173b) seems to be the anomaly in this case as a Cbz group should be more lipophilic than the ethoxycarbonyl group of (173a). However, Cbz groups in this position in A-74704 (34, Fig. 17) have been shown to lie along the plane of the main chain of the inhibitor instead of in one of the hydrophobic pockets of the HIV protease site, therefore reducing the contacts between the main chain of the inhibitor and the protease site reducing efficacy.

A possible schematic diagram of the hydrogen bonding of steroids (168), (173a), (173b) and (173c) in the HIV protease site is shown in Fig 59.
Figure 59 - A possible general scheme of hydrogen bonding between HIV-Pr and steroidal inhibitors. We have used the nomenclature of Schechter and Berger\(^{303}\) for the subsites upon the enzyme.

To gain some insight about the interactions of the compounds (168), (173a), (173b) and (173c) with the protease enzyme, the general scheme Fig. 56 has been suggested when compared with A74704 (34, Fig. 17).

The binding of the inhibitors involve a number of crucial hydrogen bonding interactions which are strongly conserved among protease-inhibitor structures:

1. those of the carbonyl which is centred between the two aspartyl residues,

2. the hydrogen bonds formed between the amide / carbamate carbonyl group between P2 / P1 residue with a conserved water molecule which bridges the carbonyl oxygen of the inhibitor with the amide hydrogens of residues Ile-50 and Ile-50' in the flap and,
All four potential inhibitors (168), (173a), (173b) and (173c) showed activity against HIV-1 infected cells. The pivaloyl derivatised enamine (173c) proved to be the most potent.

This result was consistent with the original molecular modelling predictions, having a bulky hydrophobic substituent on C-11 nitrogen function to fit into the P1 subsite of the HIV-1 protease enzyme (Fig. 59).

This series of inhibitors has potential to be derivatised elsewhere in the molecule (eg aromatisation of the A-ring) for further studies in the future.
4.2 Concluding Remarks.

The design and synthesis of novel steroidal inhibitors of HIV-1 protease has been described. Enamines (168), (173a), (173b) and (173c) derived from deoxycholic acid have been synthesised and show modest activity against the HIV-1 virus.

Dehydrogenation of C-3 keto and / or C-12 keto compounds using BSA or its catalytic in situ method has proven to be effective in producing 1,4-dien-3-one systems.

Successful epoxidation has been achieved in various Δ11-deoxycholic acid derivatives with conventional (mCPBA) methods and the in situ dioxirane method.

Nitrogen nucleophiles were unsuccessful in providing the corresponding target amino-alcohols from 11α,12α-epoxides. However, ring opening was possible, giving rearranged products.

A number of A-ring steroidal 1,4-dien-3-one systems have been aromatised utilising activated zinc in ‘wet’ pyridine. The products depend upon the position and type of C-ring substituents and provide new evidence in support of a radical anion mechanism. This pathway shows potential for the synthesis of estrogenic HIV-1 protease inhibitors in the future.
Chapter 5.
5.1 General Experimental.

Commercially available solvents and reagents were utilised throughout without further purification, other than those detailed below. 'Light Petroleum' refers to the fraction of petroleum ether boiling between 40 °C and 60 °C and was distilled over calcium chloride through a 36 cm Vigreux column before use. Dichloromethane was purified and dried by distillation over phosphorous pentoxide. Toluene was distilled over calcium hydride before being stored under nitrogen and over activated 4Å molecular sieves. Tetrahydrofuran was distilled from sodium benzophenone ketyl whilst under nitrogen prior to use. Methanol was distilled from dried magnesium and iodine. Pyridine was distilled from potassium hydroxide pellets and stored over 4Å sieves. Analytical thin layer chromatography (TLC) was carried out using aluminium backed plates coated with Merck Kieselgel 60 GF254. All developed plates were visualised under ultra-violet light at 254 and 360nm and/or by staining with molybdate and permanganate dips. Flash chromatography was carried out using Merck Kieselgel 60H silica and pressure was applied to the separation column either pre-adsorbed onto silica or as a saturated solution in an appropriate solvent.

Infrared spectra were recorded in the range 4000 - 600 cm⁻¹ using a Nicolet FT-205 spectrometer with internal calibration. Spectra were recorded as either solutions in dichloromethane or chloroform of deuterochloroform, as thin films or as Nujol mulls. Thin film and Nujol mull spectra were recorded using sodium chloride plates. ¹H NMR spectra were recorded using Bruker AC-250 (250 MHz) and Bruker-400 (400 MHz) instruments. ¹³C NMR spectra were recorded on Bruker AC-250 (62.9 MHz) and Bruker-400 (100.6 MHz) instruments. ¹H NMR spectra are referenced against tetramethylsilane at 0.00 ppm and deuterated chloroform 7.27 ppm. Chemical shift values δₘ and δₐ are accurate to ± 0.01 ppm. Signals are described as being broad (b), singlets (s), doublets (d), triplets (t), quartets (q) and multiplets (m). High resolution mass spectra were recorded on a Kratos MS80 instrument or on a VAB-E instrument (Swansea EPSRC mass spectrometry service) by Electron
Impact (EI) or Chemical Ionisation (CI). Melting points were recorded on a Koffler hotplate stage apparatus and are uncorrected. Optical rotations were recorded upon POLAR 2001 Polarimeter in an appropriate solvent.

Parts of this work have been repeat experiments of references 214, 272, 273 and were researched in collaboration with Dr. G. C. Loftus and are marked as such.
5.2 Experimental.

Methyl 3α,12α-dihydroxy-5β-cholan-24-oate (98, R = OMe)

Deoxycholic acid (97, 11.57 g, 29.47 mmol) in THF (100 cm$^3$) at 0 °C was treated dropwise with freshly prepared diazomethane$^{122}$ (33 mmol) in diethyl ether until a yellow colour persisted. After 5 h at 0 °C, glacial acetic acid (0.1 cm$^3$) was added to decompose any excess diazomethane. The solvent was removed in vacuo to yield a white foam (12.23 g). Purification using flash chromatography (silica gel, 10 : 10 : 1 EtOAc / CH$_2$Cl$_2$ / AcOH) gave the title compound (11.86 g, 99 %) as colourless needles.

Mpt. 95 - 97 °C [lit.$^{304}$ mpt. 95 - 97 °C]; IR (CH$_2$Cl$_2$) $\nu_{\text{max}}$ (cm$^{-1}$) 3386 (OH), 1738 (C24 C=O); $^1$H NMR (CDCl$_3$, 250 MHz) $\delta$ 0.67 (s, CH$_3$-18, 3H), 0.82 (s, CH$_3$-19, 3H), 0.96 - 0.98 (d, J = 6 Hz, CH$_3$-21, 3H), 3.20 - 3.70 (m, CH-3β, 1H), 3.65 (s, OCH$_3$, 3H), 3.97 (s, CH-12β, 1H); $^{13}$CNMR (CDCl$_3$, 62.9 MHz) 12.46 (C-18), 17.22 (C-21), 23.07 (C-19), 23.57 (C-15), 26.04 (C-16), 26.52 (C-7), 27.37 (C-6), 28.58 (C-11), 30.38 (C-2), 30.82 (C-22), 31.00 (C-23), 33.56 (C-9), 34.53 (C-10), 35.04 (C-20), 35.14 (C-1), 35.95 (C-8), 36.33 (C-4), 41.99 (C-5), 46.41 (C-13), 47.22 (C-17), 48.48 (C-14), 51.43 (OMe), 71.71 (C-3), 73.06 (C-12), 174.64 (C-24); [$\alpha$]$_D^{20}$ = + 25° (0.050 gml$^{-1}$, MeOH). [Found M$^+$ 406.3083 (1 %), 388 (14, - H$_2$O), 370 (30, - H$_2$O) 273 (100) Calc. for C$_{25}$H$_{42}$O$_4$ M, 406.3083].
Benzylaminodeoxycholic acid (98, \( R = \text{HN-CH}_2\text{-Ph} \))

A suspension of benzylamine hydrochloride (97, 1.01 g, 7 mmol) in 70 cm³ of ethyl acetate containing 1 cm³ of triethylamine was stirred at room temperature for 1 h. Deoxycholic acid (98, \( R = \text{OMe} \), 1.96 g, 5 mmol) and EEDQ (\( N \)-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, 1.73 g, 7 mmol) were then added to the solution. After stirring for a further 10 minutes, the suspension was refluxed overnight. The crystalline suspension was filtered and washed with water (50 cm³) and ethyl acetate (10 cm³). The ethyl acetate layer was separated, washed successively with 0.5 M NaOH (50 cm³), water (50 cm³) and 0.5 M HCl (2 x 50 cm³) then concentrated in vacuo to give a colourless solid. Recrystallisation from methanol gave the title compound (2.24 g, 93 %) as colourless needles.

Mpt. 180 - 182 °C; IR (CH₂Cl₂)\( \nu_{\text{max}} \) (cm⁻¹) 3395 (OH), 3314 (NH), 3078 (CH Aromatic), 2926 (CH Aliphatic), 1648 (C=O); \(^1\)H NMR (400 MHz, CDCl₃) \( \delta \) 0.67 (s, \text{CH}_3-18, 3H), 0.90 (s, \text{CH}_3-19, 3H), 0.97 - 0.98 (d, \( J = 6 \) Hz, \text{CH}_3-21, 3H), 3.61 (m, CH-3, 1H), 3.96 (s, CH-12, 1H), 4.42 - 4.44 (q, \( J = 6 \) Hz, HNC\text{H}_2\text{-Ph}, 2H), 5.83 (b. s, NH, 1H), 7.25 - 7.29 (m, Ar-H, 5H); \(^{13}\)C NMR (100 MHz, CDCl₃) 12.73 (C-18), 17.38 (C-21), 23.12 (C-19), 23.70 (C-15), 26.14 (C-16), 27.14 (C-7), 27.38 (C-6), 28.54 (C-11), 31.66 (C-2), 32.52 (C-22), 33.38 (C-23), 33.57 (C-9), 34.13 (C-10), 35.26 (C-20), 35.27 (C-1), 35.99 (C-8), 36.28 (C-4), 42.06 (C-5), 43.59 (NH-CH₂-Ph), 46.48 (C-13), 47.00 (C-17), 48.16 (C-14), 71.74 (C-3), 73.23 (C-12), 127.54 (m-Ar-C), 127.86 (o-Ar-C), 128.71 (p-Ar-C), 138.42 (i-Ar-C), 173.80 (CO-NH). [Found MH⁺ 482.3634 (100 %), 464 (11), 392 (8), 108 (3) \( \text{C}_{31}\text{H}_{47}\text{N}_{03} \) requires MH, 482.3634].
Glycodeoxycholic acid ethyl ester (98, R = NH-CH₂-CO₂C₂H₅)

A suspension of ethyl glycinate hydrochloride (0.98 g, 7 mmol) in 70 cm³ of ethyl acetate containing 1 cm³ of triethylamine was stirred at room temperature for 1 h. Deoxycholic acid (97, 1.96 g, 5 mmol) and EEDQ (N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, 1.73 g, 7 mmol) were then added to the solution. After stirring for a further 10 minutes, the suspension was then refluxed overnight. The suspension was filtered and washed with water (50 cm³) and ethyl acetate (10 cm³). The ethyl acetate layer was separated, washed successively with 0.5 M NaOH (50 cm³), water (50 cm³), 0.5 M HCl (2 x 50 cm³) and concentrated in vacuo to give a colourless solid. Recrystallisation from methanol gave the title compound (2.21 g, 92 %) as colourless needles.

Mpt. 189 - 190 °C; IR (CHCl₃) $\nu_{\text{max}}$ (cm⁻¹) 3364 (OH), 3079 (NH); 3037 (Aliphatic CH), 1740 (C=O Glycine ethyl ester), 1658 (C=O); $^1$H NMR (400 MHz, CDCl₃) δ 0.60 (s, CH₂-18, 3H), 0.83 (s, CH₃-19, 3H), 0.91 - 0.93 (d, J = 6 Hz, CH₂-21, 3H), 1.20 - 1.21 (t, J = 3 Hz, OCH₂CH₃, 3H), 2.50 (b. s, OH, 2H), 3.53 (m, CH-3β, 1H), 3.90 (s, CH-12β, 1H), 3.94 - 3.95 (q, J = 5 Hz, NHC₂H₂CO, 2H), 4.11 - 4.16 (q, J = 7 Hz, OCH₂CH₃, 2H), 8.27 (s, NH, 1H); $^{13}$C NMR (100 MHz, CDCl₃) 14.57 (C-18), 15.97 (CH₂CH₃), 19.25 (C-21), 24.98 (C-19), 25.55 (C-15), 28.02 (C-16), 29.03 (C-7), 29.36 (C-6), 30.50 (C-11), 32.28 (C-2), 33.30 (C-22), 34.92 (C-23), 35.47 (C-9), 35.97 (C-10), 37.12 (C-20), 37.14 (C-1), 37.89 (C-8), 38.27 (C-4), 43.19 (OCH₂CH₃), 43.97 (C-5), 48.36 (C-13), 48.88 (C-17), 50.03 (C-14), 63.28 (NH-CH₂-CO), 73.53 (C-3), 74.93 (C-12), 172.13 (CO₂C₂H₅), 175.27 (CONH). [Found MH⁺ 478.3532 (98 %), 460 (48), 385 (29), 193 (31), C₂₈H₄₇NO₅ requires MH, 478.3533].
Methyl 3α-acetyl-12α-hydroxy-5β-cholan-24-oate (99a) and Methyl 3α,12α-diacteoy-5β-cholan-24-oate (99b).

Acetic anhydride (0.28 g, 2.71 mmol) was added dropwise to a solution of methyl 3α,12α-dihydroxy-5β-cholan-24-oate (98 R = OMe, 1.00 g, 2.46 mmol) in anhydrous pyridine over 2 h. After stirring for 16 h at room temperature, the solution was poured over iced water (200 cm$^3$) and extracted into CH$_2$Cl$_2$ (3 x 50 cm$^3$). The combined extracts were washed with HCl (2M, 50 cm$^3$), H$_2$O (50 cm$^3$), sat. NaHCO$_3$ (50 cm$^3$), dried over MgSO$_4$ and concentrated in vacuo. Purification using flash chromatography (silica gel, 50 - 70 % light petrol / diethyl ether) gave the title compounds as a colourless solids. Recrystallisation from MeOH gave the title compounds (99a, 0.467 g, 42%), (99b, 0.267 g, 22 %) both as colourless needles.

(99a) Mpt. 130 - 132 °C [lit.$^{305}$ mpt. 133 - 135 °C]; IR (nujol) $\nu_{\text{max}}$ (cm$^{-1}$) 3453 (OH), 1736 (C24 C=O); $^1$H NMR (250 MHz, CDCl$_3$) $\delta$ 0.68 (s, CH$_3$-18, 3H), 0.92 (s, CH$_3$-19, 3H), 0.96 - 0.99 (d, J = 6 Hz, CH$_3$-21, 3H), 2.02 (s, CH$_3$CO, 3H), 3.67 (s, OCH$_3$, 3H), 3.98 (s, CH-12β, 1H), 4.75-4.85 (m, CH-3β, 1H);

$^{13}$C NMR (100 MHz, CDCl$_3$) 13.86 (C-18), 18.92 (C-21), 22.85 (C3-CH$_3$CO), 24.51 (C-19), 24.85 (C-15), 27.07 (C-16), 27.28 (C-7), 28.31 (C-2), 28.77 (C-11), 29.52 (C-6), 29.64 (C-23), 32.01 (C-22), 32.40 (C-4), 33.46 (C-1), 35.46 (C-8), 35.61 (C-10), 35.81 (C-20), 36.08 (C-9), 37.08 (C-5), 43.23 (C-13), 46.43 (C-17), 49.99 (C-14), 50.85 (OMe), 74.72 (C-3), 75.70 (C-12), 170.14 (C3-CH$_3$C=O), 174.56 (C-24).

(99b) Mpt. 117 - 119 °C [lit.$^{301}$ mpt. 118 - 119 °C]; IR (nujol) $\nu_{\text{max}}$ (cm$^{-1}$) 2949 (CH Aliphatic), 1735 (C24 C=O); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.72 (s, CH$_3$-
18, 3H), 0.80 - 0.81 (d, J = 6 Hz, CH₃-21, 3H), 0.91 (s, CH₃-19, 3H), 2.04 (s, C₃-CH₃CO, 3H), 2.10 (s, C-12-CH₃CO, 3H), 3.66 (s, OCH₃, 3H), 4.67 - 4.73 (m, CH-3β, 1H), 5.31 (s, CH-12β, 1H); ¹³C NMR (100 MHz, CDCl₃) 12.40 (C-18), 17.50 (C-21), 21.40 (C₃-CH₃CO), 21.49 (C₁₂-CH₃CO), 23.08 (C-19), 23.43 (C-15), 25.64 (C-16), 25.85 (C-7), 26.69 (C-2), 26.71 (C-11), 26.79 (C-6), 27.71 (C-23), 30.82 (C-22), 30.98 (C-4), 32.17 (C-1), 34.03 (C-10), 34.39 (C-8), 34.70 (C-20), 35.65 (C-9), 41.81 (C-5), 44.99 (C-13), 47.57 (C-17), 49.43 (C-14), 51.54 (OMe), 74.21 (C-3), 75.91 (C-12), 170.53 (C₃-CH₃CO₂), 170.61 (C₁₂-CH₃CO₂), 174.65 (C-24).

Methyl 3α-t-butyldimethylsilyloxy-12α-hydroxy-5β-cholan-24-oate (99c)

Methyl deoxycholate (98, R = Me, 0.50 g, 1.23 mmol) and imidazole (92 mg, 1.35 mmol) were dissolved in DMF (50 cm³) under nitrogen. TBDMSCI (0.20 g, 1.35 mmol) was added to the reaction mixture, vigorously stirring at room temperature overnight. The reaction mixture was then quenched with water (100 cm³) and extracted into ethyl acetate (3 x 50 cm³). The combined organic phase was washed with water (100 cm³), sat. NaCl solution (100 cm³), water (100 cm³), dried over MgSO₄ and finally concentrated in vacuo to give a pale yellow solid. The product was purified by recrystallisation from ethyl acetate to afford colourless needles of the title compound (0.47 g, 73 %).

Mpt. 104 - 105 °C; IR (CH₂Cl₂) νmax (cm⁻¹) 3486 (OH), 1739 (C=O); ¹H NMR (400 MHz, CDCl₃) δ 0.05 (s, Si(CH₃)₂, 6H) 0.67 (s, CH₃-18, 3H), 0.87 (s, SiC(CH₃)₃, 9H), 0.88 (s, CH₃-19, 3H), 0.96 - 0.98 (d, J = 6 Hz, CH₃-21, 3H), 3.53 - 3.62 (m, CH-3β, 1H), 3.67 (s, OCH₃, 3H), 3.96 (s, CH-12β, 1H);
13C NMR (100 MHz, CDCl₃) -4.60 (SiCH₃), 12.73 (C-18), 17.28 (C-21), 18.27 (C(CH₃)₃), 23.23 (C-19), 23.64 (C-15), 25.96 (C(CH₃)₃), 26.10 (C-16), 27.42 (C-7), 27.44 (C-6), 28.26 (C-11), 29.79 (C-2), 30.87 (C-22), 30.90 (C-23), 31.05 (C-9), 33.63 (C-20), 34.15 (C-10), 35.08 (C-8), 36.04 (C-1), 37.14 (C-4), 42.24 (C-5), 46.46 (C-13), 47.24 (C-17), 48.20 (C-14), 51.52 (OMe), 72.75 (C-3), 73.19 (C-12), 74.76 (C-24).

[Found M-^tBuMe₂SiO-H₂O^+ 371.2948 (70%), 255 (35), 149 (65), 104 (30)
Calc. for C₂₅H₃₉O₂ M-^tBuMe₂SiO-H₂O, 371.2950].

Methyl 3α-benzoyl-12α-hydroxy-5β-cholan-24-oate (99d) and Methyl 3α,12α-dibenzyloxy-5β-cholan-24-oate (97e).

A solution of benzoyl chloride (0.38 g, 2.71 mmol) in CH₂Cl₂ (10 cm³) was added dropwise to a mixture of pyridine (50 cm³) in CH₂Cl₂ (50 cm³) at 0 °C. To the resulting benzoylating agent, methyl deoxycholate (98, R = OMe, 1.00 g, 2.46 mmol) was added portionwise, maintaining the reaction temperature below 10 °C. The reaction was then stirred for a further 24 h at 0 °C. The solution was then poured over iced water (200 cm³) and extracted into CH₂Cl₂ (3 x 50 cm³). The combined extracts were washed with HCl (2M, 50 cm³), H₂O (50 cm³), sat. NaHCO₃ (50 cm³), dried over MgSO₄ and concentrated in vacuo. Purification using flash chromatography (silica gel, 3 : 1 light petrol / diethyl ether) and then recrystallisation from MeOH gave the title compounds (99d, 1.06 g, 84 %), (99e, 35 mg, 2 %), both as colourless needles.
(99d) Mpt. 96 - 98°C [lit. \(96^\circ\text{C}\); IR (CH\(_2\)Cl\(_2\)) \(\nu_{\text{max}}\) (cm\(^{-1}\)) 3395 (OH), 1739 (C=O) 1716 (PhCO\(_2\)); \(^1\)H NMR (250 MHz, CDCl\(_3\)) \(\delta\) 0.62 (s, CH\(_3\)-18, 3H), 0.88 (s, CH\(_3\)-19, 3H), 0.90 - 0.92 (d, \(J = 6\) Hz, CH\(_3\)-21, 3H), 3.66 (s, OCH\(_3\), 3H), 4.80 - 5.01 (m, CH-3\(\beta\), 1H), 7.31 - 7.35 (m, Ar-H (meta), 2H), 7.37 - 7.47 (m, Ar-H (para), 1H), 7.94 - 8.05 (m, Ar-H (ortho), 2H); \(^{13}\)C NMR (62.9 MHz, CDCl\(_3\)) 12.68 (C-18), 17.28 (C-21), 23.08 (C-19), 23.52 (C-15), 25.95 (C-16), 26.58 (C-4), 26.91 (C-7), 27.36 (C-6), 28.67 (C-11), 30.82 (C-2), 30.96 (C-22), 32.23 (C-23), 33.67 (C-9), 34.12 (C-10), 34.86 (C-1), 34.99 (C-20), 35.94 (C-8), 41.87 (C-5), 46.43 (C-13), 47.30 (C-17), 48.26 (C-14), 51.54 (OMe), 73.16 (C-3), 74.79 (C-12), 128.36 (m-Ar-C), 129.47 (o-Ar-C), 130.78 (i-Ar-C), 132.60 (p-Ar-C), 166.90 (PhCO\(_2\)), 174.65 (C-24); \([\alpha]_D^{20} = +61^{\circ} (0.05\ \text{g}\cdot\text{mL}^{-1}, \text{methanol}). \) [Found M\(^+\) 510.3340 (1 %), 508 (8), 388 (15), 370 (28) Calc. for C\(_{32}\)H\(_{46}\)O\(_8\)M, 510.3345].

(99e) Mpt. 143 - 144 °C [lit. \(145 - 146\) °C]; IR (CH\(_2\)Cl\(_2\)) \(\nu_{\text{max}}\) (cm\(^{-1}\)) 3062 (CH Aromatic), 2948 (CH Aliphatic), 1737 (C=O), 1715 (PhCO\(_2\)); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.82 (s, CH\(_3\)-18, 3H), 0.83 - 0.85 (d, \(J = 6\) Hz, CH\(_3\)-21, 3H), 0.96 (s, CH\(_3\)-19, 3H), 3.61 (s, OCH\(_3\), 3H), 4.80 - 4.92 (m, CH-3\(\beta\)), 5.39 (s, CH-12\(\beta\), 1H), 7.37 - 7.41 (m, Ar-H, 2H), 7.47 - 7.58 (m, Ar-H, 4H), 7.66 (m, Ar-H, 2H), 8.09 - 8.12 (m, Ar-H, 2H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) 12.57 (C-19), 17.51 (C-21), 23.02 (C-18), 23.54 (C-15), 25.75 (C-16), 26.06 (C-4), 26.38 (C-7), 26.91 (C-6), 27.46 (C-11), 30.82 (C-2), 31.02 (C-22), 32.23 (C-23), 34.02 (C-12), 34.60 (C-9), 34.74 (C-20), 34.83 (C-1), 35.73 (C-8), 41.72 (C-5), 45.53 (C-13), 47.92 (C-17), 50.20 (C-14), 51.45 (OMe), 74.49 (C-3), 76.50 (C-12), 128.13 (3 - Benzoate m-Ar-C), 128.52 (12 - Benzoate m-Ar-C), 129.48 (2 x o-Ar-C), 130.82 (3 - Benzoate i-Ar-C), 130.96 (12 - Benzoate i-Ar-C), 132.62 (3 - Benzoate p-Ar-C), 132.77 (12 - Benzoate p-Ar-C), 165.82 (3 - Benzoate PhCO\(_2\)), 166.93 (12 - Benzoate PhCO\(_2\)), 174.57 (C-24). [Found M-C\(_{14}\)H\(_{10}\)O\(_4^+\) 372.3041 (5 %, - 2 x PhCO\(_2\)), 370 (87), 355 (12), 255 (77) Calc. for C\(_{25}\)H\(_{40}\)O\(_2\)M-C\(_{14}\)H\(_{10}\)O\(_4\), 372.3028].
3α-Benzyloxy-benzylaminodeoxycholic acid (99f)

A solution of benzoyl chloride (0.16 g, 1.14 mmol) in CH₂Cl₂ (5 cm³) was added dropwise to a mixture of pyridine (25 cm³) in CH₂Cl₂ (25 cm³) at 0 °C. To the resulting benzoylating agent, benzylaminodeoxycholic acid (98, R = HN-CH₂-Ph, 0.50 g, 1.04 mmol) was added portionwise, maintaining the reaction temperature below 10 °C. The reaction was then stirred for a further 24 h at 0 °C. The solution was then poured over iced water (100 cm³) and extracted into CH₂Cl₂ (3 x 25 cm³). The combined extracts were washed with HCl (2M, 50 cm³), H₂O (50 cm³), sat. NaHCO₃ (50 cm³), dried over MgSO₄ and concentrated in vacuo. Purification using flash chromatography (silica gel, 4 : 1 light petrol / diethyl ether) and then recrystallisation from MeOH gave the title compound (99f, 0.41 g, 68 %) as colourless prisms.

Mpt. 108 - 110 °C; IR (CH₂Cl₂) νmax (cm⁻¹) 3413 (OH), 3302 (NH), 3051 (C-H Aromatic), 2934 (CH Aliphatic) 1686 (C=O); ¹H NMR (400 MHz, CDCl₃) δ 0.70 (s, CH₃-18, 3H), 0.97 (s, CH₃-19, 3H), 1.01 - 1.00 (d, J = 6 Hz, CH₃-21, 3H), 4.01 (s, CH-12β, 1H), 4.44 - 4.46 (q, J = 6 Hz, HNCH₂Ph, 2H), 4.96 - 5.01 (m, CH-3β, 1H), 5.98 (b. s, NH, 1H), 7.28 - 7.30 (m, Ar-H, 5H), 7.42 (m, Ar-H, 2H), 7.46 (m, Ar-H, 1H), 8.04 - 8.07 (m, Ar-H, 2H); ¹³C NMR (100 MHz, CDCl₃) 12.76 (C-18), 17.51 (C-21), 23.22 (C-19), 24.12 (C-15), 25.98 (C-16), 27.03 (C-7), 27.38 (C-6), 27.78 (C-11), 28.38 (C-2), 31.02 (C-9), 32.49 (C-22), 33.28 (C-23), 34.68 (C-1), 36.01 (C-10), 36.25 (C-4), 36.71 (C-20), 42.13 (C-5), 43.55 (NH-CH₂Ph), 44.36 (C-8), 47.12 (C-17), 48.30 (C-14), 48.46 (C-13), 72.86 (C-3), 73.25 (C-12), 126.89 (m-Ar-C Benzyamine), 128.11 (o-Ar-C Benzyamine), 128.36 (p-Ar-C Benzyamine), 128.49 (m-Ar-C Benzoate), 129.72 (o-Ar-C Benzoate), 130.53 (i-Ar-C Benzoate), 132.87 (p-
Ar-C Benzoate), 137.21 (i-Ar-C Benzylamine), 166.01 (PhC0 2 ), 173.29 (C-24). [Found MH+ 586.3900 (100 %), 568 (21), 552 (23), 524 (24) C38H52NO4 requires MH, 586.3896].

3α-Benzylxy-glycodeoxycholic acid ethyl ester (99g)

A solution of benzoyl chloride (0.16 g, 1.15 mmol) in CH2Cl2 (5 cm3) was added dropwise to a mixture of pyridine (25 cm3) in CH2Cl2 (25 cm3) at 0 °C. To the resulting benzoylating agent, benzylaminodeoxycholic acid (98, R = HN-CH2-Ph, 0.50 g, 1.05 mmol) was added portionwise, maintaining the reaction temperature below 10 °C. The reaction was then stirred for a further 24 h at 0 °C. The solution was then poured over iced water (100 cm3) and extracted into CH2Cl2 (3 x 25 cm3). The combined extracts were washed with HCl (2M, 50 cm3), H2O (50 cm3), sat. NaHCO3 (50 cm3), dried over MgSO4 and concentrated in vacuo. Purification using flash chromatography (silica gel, 4 : 1 light petrol / diethyl ether) gave the title compound (99f, 0.43 g, 71 %) as a colourless foam.

IR (CDCl3) \( \nu_{\text{max}} \) (cm\(^{-1}\)) 3375 (OH), 3319 (NH), 3062 (CH Aromatic), 2938 (CH Aliphatic), 1740 (C=O glycine ethyl ester) 1715 (PhC0 2 ) 1659 (C24 C=O); 1H NMR (400 MHz, CDCl3) 6 0.61 (s, CH3-18, 3H), 0.91 (s, CH3-19, 3H), 0.91-0.92 (d, J = 6 Hz, CH3-21, 3H), 1.16 - 1.21 (t, J = 2 Hz, OCH2CH3, 3H), 2.90 (b. s, OH, 2H), 3.93 - 3.94 (q, J = 5 Hz, NH-CH2-CO ,CH-12β, 3H), 4.09-4.14 (q, J = 7 Hz, OCH2CH3, 2H), 4.88 (m, CH-3β, 1H), 6.40 (b. s, NH, 1H), 7.32 - 7.36 (m, Ar-H, 2H), 7.44 - 7.48 (m, Ar-H, 1H), 7.94 - 7.96 (m, Ar-
H, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) 12.64 (C-18), 14.04 (CH$_3$CH$_2$O), 17.30 (C-21), 23.05 (C-19), 23.55 (C-15), 25.95 (C-16), 26.26 (C-7), 26.85 (C-6), 27.40 (C-11), 28.42 (C-2), 31.37 (C-22), 32.88 (C-23), 33.58 (C-9), 34.08 (C-10), 34.16 (C-20), 35.12 (C-4), 35.17 (C-8), 35.87 (C-1), 41.21 (OCH$_2$CH$_3$), 41.85 (C-5), 46.38 (C-13), 46.93 (C-17), 48.14 (C-14), 61.35 (NH-C$_2$H$_2$-CO), 72.92 (C-3), 74.88 (C-12), 128.15 (m-Ar-C), 129.41 (o-Ar-C), 130.69 (i-Ar-C), 132.62 (p-Ar-C), 166.09 (PhCO$_2$), 170.20 (CO$_2$C$_2$H$_5$), 174.00 (C-24). [Found MH$^+$ 582.3795 (84 %), 564 (72), 442 (82), 385 (100) requires C$_{35}$H$_{52}$NO$_6$ MH, 582.3795].

Methyl 3$\alpha$-tert-butyldimethylsilyloxy-12-oxo-5$\beta$-cholan-24-oate (100a)

To a solution of methyl 3$\alpha$-tert-butyldimethylsilyloxy-12$\alpha$-hydroxy-5$\beta$-cholan-24-oate (99c, 0.10 g, 0.27 mmol) at 0 °C in acetone (10 cm$^3$) was added Jones' reagent (0.30 mmol) dropwise with vigorous stirring under nitrogen. The mixture was allowed to react for 25 minutes after which the reaction was quenched with distilled water (90 cm$^3$) and the mixture extracted with diethyl ether (3 x 25 cm$^3$). The combined extracts were washed with sat. NaHCO$_3$ (3 x 25 cm$^3$), dried over MgSO$_4$ and the solvent removed in vacuo to give a colourless gum (0.07 g). Purification by recrystallisation from methanol yielded white prisms of the title compound (42 mg, 42 %)

Mpt. 99 - 100 °C; IR (CH$_2$Cl$_2$) $\nu_{\text{max}}$ (cm$^{-1}$) 1734 (C=O), 1712 (PhCO$_2$); $^1$H NMR (250 MHz, CDCl$_3$) $\delta$ 0.03 (s, Si(CH$_3$)$_2$, 6H), 0.99 - 1.03 (d, J = 6 Hz, CH$_3$-21, 3H), 1.06 (s, CH$_3$-18, SiC(CH$_3$)$_3$, 12H), 1.08 (s, CH$_3$-19, 3H), 3.50-3.60 (m, CH-3$\beta$, 1H), 3.66 (s, OCH$_3$, 3H); $^{13}$C NMR (62.9 MHz, CDCl$_3$) -4.58 (SiCH$_3$), 12.56 (C-18), 17.08 (C-21), 18.24 (C(CH$_3$)$_3$), 23.01 (C-19), 23.54
Methyl 3α-benzyloxy-12-keto-5β-cholan-24-oate (100b)

To a rapidly stirred solution of sodium dichromate (0.40 g, 1.71 mmol) in distilled water (5 cm³) was added dropwise concentrated sulphuric acid (0.5 cm³, 98%). The resulting chromic acid was added to methyl 3α-benzyloxy-12α-hydroxy-5β-cholan-24-oate (99d, 1.00 g, 1.96 mmol) in diethyl ether (30 cm³) at 0 °C and then stirred for 90 minutes. The two-phase mixture was separated, the aqueous layer extracted with diethyl ether (2 x 25 cm³), the combined organic extracts washed with saturated NaHCO₃ (3 x 30 cm³), dried over MgSO₄ and the solvent removed in vacuo to yield a colourless gum. Recrystallisation from methanol yielded the title compound as white prisms (0.86 g, 86%).

Mpt. 126 - 128 °C [lit.³⁰⁶ mpt. 128 - 129 °C]; IR (CH₂Cl₂) v_{max} (cm⁻¹) 1735 (C=O); ¹H NMR (250 MHz, CDCl₃) δ 0.85 - 0.87 (d, J = 6 Hz, CH₃-21, 3H), 1.03 (s, CH₃-18, 3H), 1.06 (s, CH₃-19, 3H), 3.67 (s, OCH₃, 3H), 4.82 - 5.23 (m, CH-3β, 1H), 7.39 - 7.55 (m, m-Ar-H, 2H), 7.52 - 7.55 (m, p-Ar-H, 1H), 8.01 - 8.04 (m, o-Ar-H, 2H); ¹³C NMR (62.9 MHz, CDCl₃) 11.62 (C-18), 18.52 (C-21), 22.70 (C-19), 24.26 (C-15), 25.95 (C-16), 26.41 (C-4), 26.89 (C-7), 27.45 (C-6), 30.45 (C-11), 31.23 (C-2), 32.18 (C-22), 34.93 (C-23),
35.43 (C-14), 35.55 (C-10), 35.61 (C-20), 36.08 (C-1), 41.34 (C-5), 44.06 (C-8), 46.40 (C-17), 51.40 (OMe), 57.47 (C-13), 58.61 (C-9), 74.20 (C-3), 128.40 (m-Ar-C), 129.45 (o-Ar-C), 130.64 (i-Ar-C), 132.68 (p-Ar-C), 166.90 (PhC=O), 174.60 (C-24), 214.73 (C-12); \([\alpha]_D^{20} = +90.8 \text{ (0.051 g mL}^{-1}, \text{ EtOH).}\]

[Found M⁺ 508.3188 (29 %), 386 (54), 271 (20), 231 (100) Calc. for C₃₂H₄₄O₅ M, 508.3188].

3α-Benzylxy-12-keto-benzylaminodeoxycholic acid (100c)

To a rapidly stirred solution of sodium dichromate (0.11 g, 0.37 mmol) in distilled water (1 cm³) was added dropwise conc. sulphuric acid (0.1 cm³, 98 %). The resulting chromic acid was added to benzylamino-3α-benzoyl-12α-hydroxy-5β-cholan-24-oate (99f, 0.2 g, 0.34 mmol) in diethyl ether (10 cm³) at 0 °C and then stirred for 90 minutes. The two-phase mixture was separated, the aqueous layer extracted with diethyl ether (2 x 10 cm³), dried over MgSO₄ and the solvent removed in vacuo to yield a colourless gum.

Purification using flash chromatography (silica, 3 : 1 light petrol : ethyl acetate) yielded the title compound as a colourless foam (0.15 g, 76 %).

IR (CDCl₃) \(\nu_{\text{max}}\) (cm⁻¹) 3373 (NH), 3062 (CH Aromatic), 2937 (CH Aliphatic), 1710 (C=O); \(^1\text{H NMR}\) (400 MHz, CDCl₃) δ 0.79 - 0.81 (d, \(J = 6\) Hz, CH₃-21, 3H), 0.98 (s, CH₃-18, 3H), 1.03 (s, CH₃-19, 3H), 1.19 - 1.23 (t, \(J = 7\) Hz, CO₂CH₂CH₃, 3H), 3.94 - 3.95 (q, \(J = 3\) Hz, NH-CH₂-CO, 2H) 4.88 (m, CH-3β, 1H), 6.08 (b, NH, 1H), 7.33 - 7.37 (m, Ar-H, 2H), 7.45 - 7.48 (m, Ar-H, 1H), 7.93 - 7.96 (m, Ar-H, 2H); \(^1\text{C NMR}\) (100 MHz, CDCl₃) 11.74 (C-18), 18.76 (C-21), 22.80 (C-19), 24.37 (C-15), 26.07 (C-4), 26.54 (C-7), 27.01 (C-6),
27.59 (C-11), 31.30 (C-2), 32.32 (C-22), 33.81 (C-23), 35.06 (C-1), 35.47 (C-10), 35.68 (C-9), 35.74 (C-20), 38.19 (C-4), 41.49 (C-5), 43.64 (NH-CH$_2$-Ph), 44.19 (C-8), 46.53 (C-17), 57.62 (C-13), 58.74 (C-14), 74.32 (C-3), 127.50 (m-Ar-C Benzylamine), 127.86 (o-Ar-C Benzylamine), 128.73 (p-Ar-C Benzylamine), 129.56 (o-Ar-C Benzoate), 130.81 (i-Ar-C Benzoate), 132.76 (p-Ar-C Benzoate), 138.53 (i-Ar-C Benzylamine), 166.10 (PhC0$_2$), 173.18 (C-24), 214.76 (C-12). [Found M$^+$ 584.3740 (92 %), 464 (70), 462 (98), 149 (43) C$_{38}$H$_{50}$NO$_4$ requires MH, 584.3740].

Methyl 3α-benzyloxy-11α-bromo-12-keto-5β-cholan-24-oate (102a).

To a solution of methyl 3α-benzyloxy-12-keto-5β-cholan-24-oate (100b, 0.15 g, 0.30 mmol), hydrogen bromide (0.2 cm$^3$, 4 M in glacial acetic acid) and glacial acetic acid (10 cm$^3$) was added bromine (52 mg, 0.32 mmol) in glacial acetic acid (5 cm$^3$) dropwise at 60°C. After stirring for 24 h the reaction mixture was poured over iced water (150 cm$^3$), extracted with diethyl ether (3 x 30 cm$^3$) and successively washed with distilled water (25 cm$^3$), saturated NaHCO$_3$ (4 x 25 cm$^3$) and distilled water (25 cm$^3$). Drying over MgSO$_4$ and removal of solvent in vacuo yielded a colourless solid (0.164 g). Purification using flash chromatography (silica, 2 : 1 light petrol / diethyl ether) afforded the title compound as colourless prisms (0.127 g, 73 %).

Mpt. 138 - 139°C; IR (CH$_3$Cl$_2$) $\nu$$_{max}$ (cm$^{-1}$) 1738 (C24 C=O), 1706 (C12 C=O); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.79 - 0.80 (d, J = 6 Hz, CH$_3$-21, 3H), 0.97 (s, CH$_3$-18, 3H), 1.16 (s, CH$_3$-19, 3H), 3.59 (s, OCH$_3$, 3H), 4.92 - 5.23 (m, CH-3β, 1H), 4.93 - 4.97 (d, J = 11 Hz, CH-11, 1H), 7.33 - 7.38 (m, m-Ar-
H, 2H), 7.45 - 7.49 (m, p-Ar-H, 1H), 7.95 - 7.97 (m, o-Ar-H 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) 11.41 (C-18), 18.52 (C-21), 23.14 (C-19), 24.98 (C-15), 26.61 (C-16), 27.34 (C-4), 27.67 (C-7), 27.84 (C-6), 30.36 (C-2), 31.19 (C-22), 33.23 (C-23), 35.38 (C-20), 37.20 (C-10), 37.21 (C-1), 38.08 (C-14), 43.79 (C-5), 48.19 (C-17), 51.49 (OMe), 51.69 (C-8), 56.29 (C-11), 57.15 (C-13), 58.63 (C-9), 74.35 (C-3), 128.27 (m-Ar-C), 129.55 (o-Ar-C), 130.78 (i-Ar-C), 132.74 (p-Ar-C), 166.13 (PhCO$_2$), 174.52 (C-24), 203.72 (C-12); $\alpha_0^{20} = +35.4$ (0.05 g mL$^{-1}$, EtOH). [Found M-Br$^+$ 507.3109 (29 %), 384 (38), 229 (81), 121 (100) C$_{32}$H$_{43}$O$_5$ requires M-Br, 507.3110].

**Methyl 3α,11α-dibenzyloxy-12-oxo-5β-cholan-24-oate (155), Methyl 3α,12β-dibenzyloxy-11-oxo-5β-cholan-24-oate (156a) and Methyl 3α,12α-dibenzyloxy-11-oxo-5β-cholan-24-oate (156b).**

To a solution of methyl 3α-benzoyl-11α-bromo-12-keto-5β-cholan-24-oate (102a, 0.03 g, 0.0051 mmol), potassium hydroxide (0.50 g, 8.91 mmol) and ethanol (90 %, 10 cm$^3$) was stirred under reflux for 24 h. The reaction mixture was then poured over iced water (50 cm$^3$), extracted with ethyl (3 x 30 cm$^3$) and successively washed with dilute HCl (0.5 M, 3 x 35 cm$^3$), distilled water (25 cm$^3$), sat. NaHCO$_3$ (4 x 25 cm$^3$) and distilled water (25 cm$^3$). Drying over MgSO$_4$ and removal of solvent in vacuo yielded a colourless solid. A solution of benzoyl chloride (1.10 g, 7.83 mmol) in CH$_2$Cl$_2$ (3 cm$^3$) was added dropwise to a mixture of pyridine (3 cm$^3$) in CH$_2$Cl$_2$ (3 cm$^3$) at 0 °C. To the resulting benzoylating agent, the colourless mixture was added portionwise, maintaining the reaction temperature above 50 °C. The reaction was then stirred for a further 24 h at room temperature.
The solution was then poured over iced water (100 cm³) and extracted into EtOAc (3 x 25 cm³). The combined extracts were washed with HCl (2M, 50 cm³), H₂O (50 cm³), sat. NaHCO₃ (50 cm³), dried over MgSO₄ and concentrated in vacuo. Purification using flash chromatography (preparative TLC, silica gel, 9:1 light petroleum : ethyl acetate) gave the title compounds in the yields as below.

(155, 6 mg, 19 %) Mpt. - Colourless prisms (MeOH) 113 - 115 °C; IR (CDCl₃) νmax (cm⁻¹) 3062 (CH Aromatic), 2949 (CH Aliphatic), 1733 (C=O), 1715 (PhCO2); ¹H NMR (400 MHz, CDCl₃) δ 0.83 - 0.84 (d, J = 6 Hz, CH₃-21, 3H), 1.19 (s, CH₃-18, 3H), 1.20 (s, CH₃-19, 3H), 3.67 (s, OCH₃, 3H), 4.99 - 5.04 (m, CH=3β, 1H), 5.73 - 5.76 (d, J = 11 Hz, CH-11β, 1H), 7.41 - 7.48 (m, Ar-H (meta), 4H), 7.54 - 7.57 (m, Ar-H (para), 2H), 8.03 - 8.09 (m, Ar-H (ortho), 2H); ¹³C NMR (100 MHz, CDCl₃) 10.93 (C-18), 18.53 (C-21), 23.15 (C-19), 24.71 (C-2), 27.28 (C-6), 27.42 (C-22), 27.49 (C-23), 30.39 (C-16), 31.18 (C-4), 32.78 (C-7), 35.35 (C-20), 35.40 (C-8), 36.75 (C-10), 37.46 (C-1), 42.90 (C-5), 46.95 (C-17), 46.99 (C-9), 51.48 (OMe), 56.82 (C-13), 57.47 (C-14), 74.40 (C-3), 76.58 (C-11), 128.27 + 128.34 (2 x m-Ar-C), 129.53 + 129.59 (2 x o-Ar-C), 130.11 + 130.75 (2 x i-Ar-C), 132.84 + 133.14 (2 x p-Ar-C), 157.33 (C-3 PhCO₂), 166.14 (C-11 PhCO₂), 174.64 (C-24), 206.63 (C-12). [Found MNH₄⁺ 646.3740 (13 %), 629 (6, MH), 528 (38), 527 (100), C₃₉H₅₂O₇N requires MNH₄⁺ 646.3740].

(156a, 10 mg, 32 %) Mpt. colourless needles (MeOH) 126 - 128 °C; IR (CDCl₃) νmax (cm⁻¹) 3062 (CH Aromatic), 2949 (CH Aliphatic), 1734 (C=O), 1715 (PhCO₂); ¹H NMR (400 MHz, CDCl₃) δ 0.82 - 0.84 (d, J = 6 Hz, CH₃-21, 3H), 0.86 (s, CH₃-18, 3H), 1.22 (s, CH₃-19, 3H), 3.65 (s, OCH₃, 3H), 4.95 - 4.99 (m, CH=3β, 1H), 5.23 (s, CH-12α, 1H), 7.42 - 7.48 (m, Ar-H (meta), 4H), 7.54 - 7.59 (m, Ar-H (para), 2H), 8.02 - 8.09 (m, Ar-H (ortho), 2H); ¹³C NMR (100 MHz, CDCl₃) 9.04 (C-18), 19.85 (C-21), 22.20 (C-19), 22.30 (C-2), 23.23 (C-15), 25.36 (C-6), 25.74 (C-22), 28.32 (C-16), 28.68 (C-
4), 31.26 (C-20), 31.45 (C-7), 33.23 (C-10), 35.52 (C-8), 41.5 (C-5), 49.37 (C-13), 49.43 (C-17), 50.49 (OMe), 52.92 (C-14), 55.23 (C-9), 73.35 (C-3), 85.32 (C-12), 127.25 + 127.41 (2 x m-Ar-C), 128.53 + 128.80 (2 x o-Ar-C), 129.78 + 130.57 (2 x i-Ar-C), 131.72 + 132.11 (2 x p-Ar-C), 164.67 (C-3 PhCO₂), 165.05 (C-12 PhCO₂), 173.28 (C-24), 202.60 (C-11). [Found MNH₄⁺ 646.3740 (82 %), 528 (31), 527 (94), 405 (48) C₃₉H₅₂O₇N requires MNH₄, 646.3740].

(156b, 6 mg, 19 %, as a colourless foam); IR (CDCl₃) ν max (cm⁻¹) 3065 (CH Aromatic), 2949 (CH Aliphatic), 1735 (C12 C=O), 1715 (PhCO₂); ¹H NMR (400 MHz, CDCl₃) δ 0.82 (s, CH₃-18, 3H), 0.83 - 0.85 (d, J = 6 Hz, CH₃-21, 3H), 0.96 (s, CH₃-19, 3H), 3.66 (s, OCH₃, 3H), 4.85 - 4.91 (m, CH-3β, 1H), 5.23 (s, CH-12β, 1H), 7.37 - 7.39 (m, Ar-H, 2H), 7.41 - 7.58 (m, Ar-H, 4H), 7.86 - 7.87 (m, Ar-H, 2H), 8.09 - 8.11 (m, Ar-H, 2H); ¹³C NMR (100 MHz, CDCl₃) 12.60 (C-18), 17.55 (C-21), 23.03 (C-19), 23.05 (C-2), 25.79 (C-15), 26.43 (C-22+C-6), 27.45 (C-23), 30.87 (C-4), 31.07 (C-16), 32.29 (C-7), 34.07 (C-13), 34.66 (C-20), 34.81 (C-1), 34.87 (C-8), 35.81 (C-5), 41.79 (C-17), 45.59 (C-10), 47.98 (C-14), 50.26 (C-9), 51.43 (OMe), 74.53 (C-3), 76.54 (C-12), 128.14 + 128.52 (2 x m Ar-C), 129.50 (2 x o-Ar-C), 130.91 + 131.05 (i-Ar-C), 132.61 + 132.75 (p-Ar-C), 165.05 (C-3 PhCO₂), 166.41 (C-12 PhCO₂), 174.56 (C-24), 202.43 (C-12). [Found MNH₄⁺ 646.3740 (100 %), 528 (28), 527 (89), 405 (46) C₃₉H₅₂O₇N requires MNH₄, 646.3740].
Methyl 11-amino-3α-benzyloxy-12-keto-5β-chol-9,11-en-24-oate (168).

To a solution of sodium azide (0.10 g, 1.54 mmol) in anhydrous DMSO (10 cm³) was added methyl 3α-benzyloxy-11β-bromo-12-keto-5β-cholan-24-oate (102a, 75 mg, 0.128 mmol) at 100 °C. After stirring for 48 h the reaction mixture was poured over ice water (50 cm³) and extracted with diethyl ether (3 x 25 cm³). The combined organic extracts were washed successively with sodium nitrite solution (25 cm³, 20%), saturated brine (25 cm³), water (25 cm³), dried over MgSO₄ and solvent removed in vacuo to yield a colourless gum (64 mg). Purification using flash chromatography (silica, 20:10:1 light petroleum / diethyl ether / triethylamine) afforded the title compound as a colourless solid (36 mg, 53%).

M.pt. 72 - 74 °C; IR (CH₂Cl₂) v max (cm⁻¹) 3512 and 3367 (NH₂), 1737 (C₂₄ C=O), 1715 (C-12 C=O), 1685 (α,β-unsat. ketone), 1600 (NH₂); ¹H NMR (400 MHz, CDCl₃) 0.88 (s, CH₃-18, 1H), 0.95-0.96 (d, J = 6 Hz, CH₃-21, 3H), 1.20 (s, CH₃-19, 3H), 3.60 (s, OCH₃, 3H), 3.80 (b, NH, 1H), 4.94 - 4.97 (m, CH-3β, 1H), 7.32 - 7.36 (m, m-Ar-H, 1H), 7.44 - 7.48 (m, p-Ar-H, 1H), 7.94 - 7.96 (m, o-Ar-H, 2H); ¹³C NMR (100 MHz, CDCl₃) 37.37 (C-18), 18.49 (C-21), 23.61 (C-15), 26.03 (C-16), 26.25 (C-7), 26.35 (C-6), 26.51 (C-19), 29.70 (C-2), 29.88 (C-23), 30.52 (C-4), 30.52 (C-22), 33.89 (C-1), 34.35 (C-20), 36.82 (C-8), 40.72 (C-10), 42.64 (C-5), 47.25 (C-17), 50.42 (C-14), 50.72 (C-13), 51.18 (OMe), 72.16 (C-3), 125.76 (C-9), 127.27 (m-Ar-C), 128.57 (o-Ar-C), 129.81 (i-Ar-C), 133.11 (p-Ar-C), 131.77 (C-11), 165.09 (PhCO₂), 173.61 (C-24), 202.18 (C-12); [α]₀ = +55.2 (0.051 g 100 mL⁻¹, EtOH). [Found M⁺ 521.3139 (100 %), 506 (18), 384 (42), 317 (22)]

C₃₂H₄₃O₅SN requires M, 521.3141.

158
Methyl 11α-azido-3α-benzyloxy-12-keto-5β-cholan-24-oate (170).

Using the same procedure as for methyl 11-amino-3α-benzoyl-12-keto-5β-chol-9,11-en-24-oate (168), except the starting methyl 3α-benzyloxy-11β-bromo-12-keto-5β-cholan-24-oate (102a) had not been purified and was used crude. The enamine (168) was isolated as usual with a second compound, the title compound present (3 mg, 4 %) isolated as a colourless foam.

IR (CDCl₃) νₘₐₓ (cm⁻¹) 3056 (CH Aromatic), 2950 (CH Aliphatic), 2103 (N 3 ), 1736 (C₂₄ C=O), 1715 (PhCO₂), 1'H NMR (400 MHz, CDCl₃) δ 0.88 - 0.90 (d, J = 6 Hz, CH₃-21, 3H), 1.03 (s, CH₃-18, 3H), 1.17 (s, CH₃-19, 1H), 3.67 (s, OCH₃, 3H), 4.02 - 4.05 (d, J = 11 Hz, CH-11β, 1H), 4.97 - 5.01 (m, CH-3β, 1H), 7.28 - 7.44 (m, Ar-H, 2H), 7.52 - 7.56 (m, Ar-H, 1H), 8.00 - 8.04 (m, Ar-H, 2H); ¹³C NMR (100 MHz, CDCl₃) 11.18 (C-18), 18.55 (C-21), 23.33 (C-19), 24.67 (C-15), 26.13 (C-16), 27.40 (C-4), 27.56 (C-7), 30.36 (C-6), 31.18 (C-2), 33.00 (C-22), 35.38 (C-23), 37.09 (C-1), 40.36 (C-10), 43.10 (C-20), 47.25 (C-14), 48.73 (C-5), 51.49 (OMe), 56.78 (C-17), 64.97 (C-11), 74.35 (C-3), 128.51 (m-Ar-C), 129.55 (o-Ar-C), 130.72 (i-Ar-C), 132.79 (p-Ar-C), 165.04 (PhCO₂), 174.50 (C-24), 208.01 (C-12). [Found MNH₄⁺ 567.3550 (32 %), 539 (16), 524 (87), 55 (71) C₃₂H₄₇N₄O₅ requires MNH₄, 567.3546].
Methyl 11α-amino-3α-benzyloxy-12-keto-5β-cholan-24-oate (171).

A solution of 0.1 g 10 % palladium-on-charcoal and methyl 11α-azido-3α-benzoyl-12-keto-5β-cholan-24-oate (170, 3 mg, 0.005 mmol) were stirred under nitrogen in anhydrous methanol (10 cm³) for 30 minutes. Hydrogen was then applied to the flask via a hydrogen balloon and the reaction mixture was stirred overnight at room temperature. After hydrogen uptake was complete by TLC the solution was passed through a short pad of silica to remove the catalyst and then evaporated to dryness yielding the title compound (2 mg, 70 %) as a colourless gum.

IR (CDCl₃) νmax (cm⁻¹) 3459 and 3358 (NH₂), 3056 (CH Aromatic), 2948 (CH Aliphatic), 1736 (C14 C=O), 1714 (PhCO₂), 1677 (C12 C=O), 1601 (NH₂);

¹HNMR (400 MHz, CDCl₃) δ 0.75 (s, CH₃-18, 1H), 0.87 - 0.89 (d, J = 6 Hz, CH₃-21, 3H), 1.04 (s, CH₃-19, 3H), 2.64 - 2.69 (dd, J = 11 and 4 Hz, CH-11β, 1H), 3.66 (s, OCH₃, 3H), 4.87 - 4.95 (m, CH-3β, 1H), 5.20 - 5.37 (b, NH, 1H), 7.39 - 7.43 (m, Ar-H, 2H), 7.51 - 7.55 (m, Ar-H, 1H), 8.02 - 8.04 (m, Ar-H, 2H). [Found MH⁺ 524.3380 (72 %), 402 (98), 225 (100), 130 (100)]

C₃₅H₄₅NO₅ requires MH⁺ 524.3378.
Methyl (N-ethoxycarbonyl)-11-amino-3α-benzyloxy-12-keto-5β-chol-9,11-en-24-oate (173a).

Methyl 11-amino-3α-benzyloxy-12-keto-5β-chol-9,11-en-24-oate (168, 0.75 g, 0.144 mmol). 4-dimethylaminopyridine (12 mg, 0.098 mmol) and ethyl chlorochromate (0.1 ~3.105 mmol) were refluxed under nitrogen for 48 h in dichloromethane (30 cm³). The resulting mixture was allowed to cool, then successively washed with HC1 (2 M, 20 cm³), saturated NaHCO₃ (20 cm³), dried over Na₂SO₄ and then concentrated in vacuo to yield a colourless gum (0.15 g). Purification using preparative TLC (silica, 5:1:1 light petroleum/dichloromethane/ EtOAc) gave the title compound as a colourless microcrystalline solid (23 mg, 27 %).

Mpt. 61 - 62 °C; IR (CH₂Cl₂) νmax (cm⁻¹) 3315 (NH), 1731 (C₂₄ C=O), 1717 (PhC₀₂), 1703 (C12 C=O); ¹H NMR (400 MHz, CDCl₃) δ 0.97 - 0.98 (d, J = 6 Hz, CH₃-21, 3H), 1.05 (s, CH₃-18, 3H), 1.21 - 1.25 (t, J = 7 Hz, CH₃-CH₂, 3H), 1.34 (s, CH₃-19, 3H), 3.66 (s, OCH₃, 3H), 4.07 - 4.15 (m, CH₃-CH₂, 2H), 5.02 - 5.05 (m, CH-3β, 1H), 5.84 (b, NH, 1H), 7.41 - 7.44 (m, m-Ar-H, 2H), 7.52 - 7.56 (m, p-Ar-H, 1H), 8.01 - 8.03 (m, o-Ar-H, 2H); ¹³C NMR (100 MHz, CDCl₃) 9.83 (C-18), 14.55 (O-CH₂CH₃), 19.14 (C-21), 24.91 (C-15), 24.91 (C-16), 26.59 (C-4), 26.64 (C-7), 27.59 (C-6), 28.02 (C-19), 29.71 (C-8), 30.63 (C-22), 30.66 (C-1), 31.43 (C-2), 34.95 (C-23), 35.45 (C-20), 38.63 (C-14), 42.56 (C-10), 47.64 (C-17), 51.47 (OMe), 52.64 (C-13), 52.82 (C-5), 61.43 (O-CH₂CH₃), 73.47 (C-3), 128.47 (C-9), 129.56 (m-Ar-C), 130.65 (o-Ar-C), 132.84 (i-Ar-C), 154.17 (C-11), 156.06 (CONH), 166.08 (p-Ar-C), 174.67 (C-24), 203.55 (C-12); [α]₂₀ = + 28° (0.017 g 100 mL⁻¹, methanol).
[Found MNH₄⁺ 611.3696 (38 %), 594 (40), 569 (12), 567 (40) C₃₅H₅₁N₂O₇ requires MNH₄, 611.3696].

**Methyl (N-benzylxoycarbonyl)-11-amino-3α-benzyloxy-12-keto-5β-chol-9,11-en-24-oate (173b).**

Methyl 11-amino-3α-benzyloxy-12-keto-5β-chol-9,11-en-24-oate (168, 75 mg, 0.144 mmol), 4-dimethylaminopyridine (12 mg, 0.098 mmol) and benzylichloroformate (0.1 cm³, 0.67 mmol) were refluxed under nitrogen for 48 h in dichloromethane (30 cm³). The resulting mixture was allowed to cool, then successively washed with HCl (2 M, 20 cm³), saturated NaHCO₃ (20 cm³), dried over Na₂SO₄ and then concentrated in vacuo to yield a colourless gum (0.12 g). Purification using preparative TLC (silica, 4:1:1 light petrol / dichloromethane / EtOAc) gave the title compound as a colourless crystalline solid (26 mg, 27 %).

Mpt. 66 - 67 °C; IR (CH₂Cl₂) νmax (cm⁻¹) 3309 (NH), 1715 (PhC=O); ¹H NMR (400 MHz, CDCl₃, 55 °C) δ 0.96-0.98 (d, J = 6 Hz, CH₃-21, 3H), 1.00 (s, CH₃-19, 3H), 1.31 (s, CH₃-18, 3H), 3.65 (s, OCH₃, 3H), 4.98-5.06 (m, CH-3β, 1H), 5.09-5.11 (d, J = 4 Hz, CH₂-Ph, 2H), 5.96 (br, NH, 1H), 7.28-7.31 (m, m-Ar-H (Cbz)), 7.38-7.42 (m, m-Ar-H, 2H), 7.49-7.51 (m, p-Ar-H, 2H), 7.98-8.00 (m, o-Ar-H, 2H); ¹³C NMR (100 MHz, CDCl₃) 9.78 (C-18), 19.14 (C-21), 24.88 (C-15), 26.57 (C-16), 26.60 (C-7) , 27.62 (C-6), 28.02 (C-19), 30.60 (C-2), 30.66 (C-23), 31.42 (C-22), 34.93 (C-4), 35.46 (C-20), 36.73 (C-1), 38.63 (C-8), 42.55 (C-10), 43.66 (C-5), 47.60 (C-17), 51.49 (OMe), 52.49 (C-14), 52.76 (C-13), 67.10 (Ph-CH₂-O), 73.42 (C-3), 127.86 (Cbz i-Ar-C), 162.
128.31 (Cbz Ar-C), 128.46 (m-Ar-C), 129.55 (o-Ar-C), 130.61 (i-Ar-C),
132.84 (p-Ar-C), 136.33 (C-9), 154.67 (CONH), 155.90 (C-11), 166.07
(PhCO₂), 174.69 (C-24), 206.91 (C-12); \([\alpha]_D^{20} = +13^\circ \) (0.028 g 100 mL⁻¹,
methanol). [Found M⁺ 655.3510 (4 %), 547 (4), 520 (6), 490 (2) C₄₀H₄₉O₇N
requires M, 655.3509].

Methyl (N-pivaloyl)-11-amino-3α-benzyloxy-12-keto-5β-chol-9,11-en-24-
oate (173c).

Methyl 11-amino-3α-benzyloxy-12-keto-5β-chol-9,11-en-24-oate (168, 75
mg, 0.144 mmol), 4-dimethylaminopyridine (12 mg, 0.098 mmol) and
trimethylacetyl chloride (0.1 cm³, 0.81 mmol) were refluxed under nitrogen
for 48 h in dichloromethane (30 cm³). The resulting mixture was allowed to
cool, then, successively washed with HCl (2 M, 20 cm³), NaHCO₃ (20 cm³),
dried over Na₂SO₄ and then concentrated in vacuo to yield a colourless gum
(0.15 g). Purification using preparative TLC (silica, 5:1:1 light petroleum /
dichloromethane / EtOAc) gave the title compound as a colourless
microcrystalline solid (0.04 g, 46 %).

Mpt. 74 - 75 °C; IR (CH₂Cl₂) \( \nu_{max} \) (cm⁻¹) 3375 (NH), 1731 (C=O), 1716
(PhCO₂), 1671 (C12 C=O); \(^1\)H NMR (400 MHz, CDCl₃) \( \delta \) 0.92 - 0.94 (d, J = 6
Hz, CH₃-21, 3H), 1.08 (s, CH₃-18, 3H), 1.22 (s, C(CH₃)₃, 9H), 1.35 (s, CH₃-
19, 3H), 3.66 (s, OCH₃, 3H), 4.83-5.23 (m, CH-3β, 1H), 6.90 (br, NH, 1H),
7.42 - 7.46 (m, m-Ar-H, 2H), 7.54 - 7.56 (m, p-Ar-H, 1H), 8.03 - 8.06 (m, o-
Ar-H, 2H); \(^13\)C NMR (100 MHz, CDCl₃) 9.95 (C-18), 18.93 (C-21), 25.00 (C-
15), 26.66 (C-4), 26.72 (C-7), 27.29 (CCH₃), 27.68 (C-6), 28.05 (C-19),

163
30.64 (C-22), 30.73 (C-1), 31.41 (C-2), 34.96 (C-23), 35.43 (C-20), 36.68 (C-16), 38.79 (C-14), 38.94 (C\textsubscript{CH\textsubscript{3}}), 42.43 (C-10), 43.73 (C-5), 47.51 (C-17), 51.46 (OMe), 52.88 (C-8), 53.01 (C-13), 73.60 (C-3), 128.33 (m-Ar-C), 128.97 (C-9), 129.57 (o-Ar-C), 130.58 (i-Ar-C), 132.89 (p-Ar-C), 152.70 (C-11), 166.09 (Ph\textsubscript{CO\textsubscript{2}}), 174.71 (C-24), 179.11 (CONH), 203.74 (C-12); \[\alpha\textsubscript{D}^\text{20}\] = +12° (0.036g 100 mL\textsuperscript{-1}, methanol). [Found MH\textsuperscript{+} 606.3800 (100 \%), 588 (4), 524 (19), 507 (8) C\textsubscript{37}H\textsubscript{52}O\textsubscript{6}N requires MH, 606.3795].

**Methyl 3α-benzyloxy-12α-mesyloxy-5β-cholan-24-oate (178).**

Methane sulphonyl chloride (2.2 cm\textsuperscript{3}, 30 mmol) in benzene (20 cm\textsuperscript{3}) was added dropwise to a stirred solution of methyl 3α-benzyloxy-12α-hydroxy-5β-cholan-24-oate (99d, 2.02 g, 4.31 mmol) in anhydrous pyridine (20 cm\textsuperscript{3}) at 0 °C. Stirring was continued for 4 h, warming gradually to room temperature. The reaction mixture was diluted with dichloromethane (100 cm\textsuperscript{3}) and washed with water (2 x 100 cm\textsuperscript{3}), 20% aqueous copper sulphate (2 x 50 cm\textsuperscript{3}), water (50 cm\textsuperscript{3}) and dried over sodium sulphate. Removal of solvent in vacuo yielded a viscous orange oil which solidified after 24 h drying under high vacuum. This product, the title compound (2.58 g, 100 \%) was used without further purification.

IR (neat) \[^{\text{v}}\text{max}\text{ (cm}^{-1}\text{)}\] 2947 (CH Aliphatic), 2870, 1740 (C=O), 1714 (Ph\textsubscript{CO\textsubscript{2}}), 1377; \[^{1}\text{H NMR (250 MHz, CDCl}\textsubscript{3}\text{)}\] \(\delta 0.78\text{ (s, CH}_3\text{-18, 3H), 0.97\text{ (s, CH}_3\text{-19, 3H), 1.01\text{ (d, J }= 6\text{ Hz, CH}_3\text{-21, 3H), 3.12\text{ (s, O}_2\text{SCH}_3\text{, 3H), 3.66\text{ (s, OCH}_3\text{, 3H), 4.95\text{ (m, CH-3β, 1H), 5.13\text{ (s, CH-12β, 1H), 7.41 - 7.68\text{ (m, m-Ar-H and p-Ar-H, 3H), 8.03 - 8.08\text{ (m, o-Ar-H, 2H).}}\text{}}}}\]
Methyl 3α-benzyloxy-5β-chol-11-en-24-oate (110, R = Me, R¹ = PhCO₂).²¹⁴

Methyl 3α-benzyloxy-12α-mesyloxy-5β-cholan-24-oate (178, 1.15 g, 1.95 mmol) was heated with potassium acetate (0.96 g, 9.78 mmol) in 1,3-dimethyl-3,4,5,6-tetrahydro-2-pyrimidone (DMPU, 11 cm³) at 125 °C for 4 h. After cooling, the reaction mixture was diluted with water (100 cm³) and extracted with dichloromethane (2 x 50 cm³). The combined organic phases were further washed with water (50 cm³) and saturated aqueous sodium chloride (50 cm³) and dried over Na₂SO₄. Removal of solvent in vacuo and purification using flash chromatography (silica gel, 1:3 diethyl ether / light petrol) afforded the title compound as colourless needles (0.88 g, 88 %).

Mpt. 103 - 106 °C; IR (CH₂Cl₂) νmax (cm⁻¹) 1731 (C₂=O), 1716 (PhC=O);¹H NMR (250 MHz, CDCl₃) δ 0.73 (s, CH₃-18, 3H), 0.92 (s, CH₃-19, 3H), 1.01 (d, δ = 6 Hz, CH₃-21, 3H), 3.65 (s, OCH₃, 3H), 4.99 (m, CH-3β, 1H), 5.43 (d, δ = 10 Hz, CH-12, 1H), 6.11 (dd, δ = 10 Hz and δ = 3 Hz, CH-11, 1H), 7.41 (m, m-Ar-H, 2H), 7.52 (m, p-Ar-H, 1H), 8.03 (m, o-Ar-H, 2H);¹³C NMR (62.9 MHz, CDCl₃) 16.59 (C-18), 18.25 (C-21), 22.91 (C-15), 23.56 (C-19), 25.40 (C-16), 26.61 (C-7), 27.87 (C-8), 28.36 (C-2), 30.80 (C-23), 30.90 (C-22), 32.90 (C-4), 34.26 (C-8), 34.83 (C-1), 34.96 (C-10), 35.87 (C-20), 40.90 (C-5), 43.04 (C-9), 44.94 (C-13), 51.37 (OMe), 51.83 (C-14), 53.50 (C-17), 74.60 (C-3), 125.28 (C-12), 128.15 (m-Ar-C), 129.42 (o-Ar-C), 130.79 (p-Ar-C) 132.58 (ρ-Ar-C), 138.78 (C-11), 165.91 (PhCO₂), 174.47 (C-24). [Found MNH₄⁺ 510.3580 (34 %), 371 (100), 255 (42) C₂₂H₄₆NO₄ requires MNH₄⁺ 510.3583].
Methyl 3α-benzylxy-11α,12α-epoxy-5β-cholan-24-oate (111, R = Me, R' = PhCO₂).

(a) A solution of methyl 3α-benzylxy-5β-chol-11-en-24-oate (110, R = Me, R' = PhCO₂, 1.16 g, 2.35 mmol) in chloroform (50 cm³) was cooled to 0 °C and treated with meta-chloroperbenzoic acid (1.60 g of 50 % m-CPBA, 4.7 mmol) and sodium carbonate (0.50 g, 4.70 mmol). After stirring at room temperature for 24 h the reaction mixture was washed with water (2 x 50 cm³) and dried over Na₂SO₄. Removal of the solvent in vacuo and purification using flash chromatography (silica, 1:2 diethyl ether / light petrol) afforded the title compound (0.80 g, 67%) as a gum.

(b) To methyl 3α-benzylxy-5β-chol-11-en-24-oate (110, R = Me, R' = PhCO₂, 0.10 g, 0.200 mmol), acetonitrile (7.5 cm³), 2,2,2-trifluoroacetophenone (0.167 g, 0.96 mmol) and (EDTA)Na₂ solution (4 x 10⁻⁴ M, 5 cm³) was added a solid mixture of Oxone® (0.59 g, 0.96 mmol) and sodium hydrogen carbonate (0.33 g, 3.10 mmol) over 30 minutes. This solution was stirred vigorously at room temperature for 24 h. After such time the reaction mixture was worked up by extraction with ethyl acetate (2 x 25 cm³), washed with saturated sodium hydrogen carbonate solution (50 cm³), dried over Na₂SO₄ and removal of solvent in vacuo to give the title compound as a colourless gum (99 mg, 96%).

IR (CH₂Cl₂) ν max (cm⁻¹) 1746 (C24 C=O); 1722 (PhCO₂); ¹H NMR (250 MHz, CDCl₃) δ 0.79 (s, CH₃-18, 3H), 1.03 (s, CH₃-19, 3H), 1.05 (d, J = 6 Hz, CH₃-21, 3H), 2.94 (d, J = 4 Hz, C11-H, 1H), 3.14 (d, J = 4 Hz, C12-H, 1H), 3.67 (s, OMe, 3H), 4.95 (m, CH-3β, 1H), 7.42 (m, m-Ar-H, 2H), 7.53 (m, p-Ar-H,
1H), 8.03 (m, o-Ar-H, 2H); 13C NMR (62.9 MHz, CDCl₃) 11.80 (C-18), 18.25 (C-21), 22.21 (C-15), 23.76 (C-19), 25.19 (C-16), 26.67 (C-7), 27.20 (C-6), 30.60 (C-23), 30.80 (C-22), 32.60 (C-4), 32.70 (C-8), 34.80 (C-1), 35.00 (C-20 & C-10), 40.90 (C-5), 41.20 (C-9), 43.40 (C-14), 46.50 (C-17), 50.30 (C-13), 51.40 (OMe), 53.40 (C-12), 60.80 (C-11), 74.60 (C-3), 128.15 (m-Ar-C), 129.42 (o-Ar-C), 130.59 (i-Ar-C), 132.58 (p-Ar-C), 165.00 (PhCO₂), 174.57 (C-24). [Found MH⁺ 509.3270 (8 %), 526 (MNH₄, 100), 508 (M, 8), 491 (M-OH, 33) C₃₂H₄₄O₅ requires MH, 509.3267].

Methyl 3α-benzylkoxy-12β-hydroxy-11-oxo-5β-cholan-24-oate (197).²¹⁴

Methyl 3α-benzylkoxy-11α,12α-epoxy-5β-cholan-24-oate (111, R = Me, R¹ = PhCO₂, 0.05 g, 0.10 mmol) was heated to 140 °C with excess sodium azide (32 mg, 0.049 mmol) in anhydrous dimethyl sulphoxide (10 cm³) containing two drops of 98 % sulphuric acid. After 16 h the reaction mixture was cooled, diluted with dichloromethane (20 cm³), washed with water (3 x 20 cm³) and aqueous saturated sodium chloride (20 cm³) and then dried over Na₂SO₄. TLC analysis indicated a single product which decomposed to a more polar second product when the crude mixture was purified using flash chromatography (silica, 1 : 2 diethyl ether / light petrol) affording the title compound as a gum (0.03 g, 57 %).

IR (CDCl₃) νmax (cm⁻¹) 3450 (OH), 1738 (C=O), 1712 (PhC=O), 1693 (C11 C=O); 'H NMR (250 MHz, CDCl₃) δ 0.61 (s, CH₃-18, 3H), 0.91-0.92 (d, J = 6 Hz, CH₃-21, 3H), 1.20 (s, CH₃-19, 3H), 2.74 (b, OH, 1H), 3.66 (s, OCH₃, 3H), 3.87 (m, CH-12α, 1H), 4.94 - 4.96 (m, CH-3β, 1H), 7.42 - 7.46 (m, m-Ar-H, 2H), 7.53 - 7.57 (m, p-Ar-H, 1H), 8.03 - 8.05 (m, o-Ar-H, 2H); 167
Methyl 3α-benzyloxy-12α-hydroxy-5β-chol-9(11)-en-24-oate (200). 214

Methyl 3α-benzyloxy-11α,12α-epoxy-5β-cholan-24-oate (111, R = Me, R′ = PhCO2, 0.01 g, 0.20 mmol) was treated with trimethylsilylazide (0.13 cm3, 0.98 mmol) in anhydrous DMF (5 cm3) for 10 minutes, then two drops of boron trifluoride etherate were added and the mixture heated for 16 h at 140 °C. The reaction mixture was then cooled, diluted with diethyl ether (50 cm3), washed with water (3 x 25 cm3) and aqueous saturated sodium chloride (25 cm3) and dried over Na2SO4. Removal of solvent in vacuo and purification by flash chromatography (silica, 1 : 1 diethyl ether / petrol) afforded the rearranged title compound as a colourless gum (5 mg, 50%).

IR (CH2Cl2) νmax (cm⁻¹) 3550 (OH), 2952 (CH Aliphatic), 2852, 1734 (C24 C=O); 1H NMR (250 MHz, CDCl3) δ 0.57 (s, CH3-18, 3H), 1.01 (d, J = 6 Hz, CH3-21, 3H), 1.12 (s, CH3-19, 3H), 3.67 (s, OCH3, 3H), 3.91 (t, collapses to a doublet on addition of D2O, J = 6 Hz, CH-3β, 1H), 4.99 (m, CH-3β, 1H), 5.66 (d, J = 6 Hz, CH-11), 7.42 (m, m-Ar-H, 2H), 7.53 (m, p-Ar-H, 1H), 8.03 (m, o-Ar-H, 2H); 13C NMR (62.9 MHz, CDCl3) 11.21 (C-18), 17.21 (C-21), 22.88 (C-15), 23.93 (C-19), 26.33 (C-16), 26.73 (C-7), 27.28 (C-6), 27.67 (C-2), 30.62 (C-23), 30.85 (C-22), 32.34 (C-4), 33.34 (C-10), 34.10 (C-1), 34.79 (C-20), 37.05 (C-8), 42.67 (C-5), 45.28 (C-9), 45.73 (C-17), 47.08 (C-14), 48.96 (C-13), 51.48 (OMe), 74.56 (C-3), 82.83 (C-12), 128.29 (m-Ar-C), 129.55 (o-Ar-C), 130.76 (i-Ar-C), 132.76 (p-Ar-C), 166.07 (PhCO2), 174.56 (C-24), 213.12 (C-11). [Found MNH4⁺ 542.3490 (76 %), 524 (M, 12), 507 (M-OH, 5), 491 (22)
C32H48NOS requires MNH4, 542.3482].
25.04 (C-15), 26.60 (C-16), 26.75 (C-7), 27.92 (C-6), 28.01 (C-2), 29.95 (C-19) 30.98 (C-23), 31.14 (C-22), 32.34 (C-4), 34.06 (C-1), 35.13 (C-20), 37.35 (C-8), 38.80 (C-10), 41.92 (C-5), 45.13 (C-13), 46.59 (C-14), 46.92 (C-17), 51.47 (OMe), 72.41 (C-3), 75.12 (C-12), 122.11 (C-11), 128.25 (m-Ar-C), 129.57 (o-Ar-C), 130.81 (i-Ar-C), 132.71 (p-Ar-C), 145.97 (C-9), 166.17 (PhC02), 174.72 (C-24). [Found M+ 508.3189 (5 %), 386 (67), 271 (17), 253 (18) C32H44O5 requires M, 508.3188].

Methyl 3α-hydroxy-5β-chol-11-en-24-oate (111, R = Me, R1 = H)

Methyl 3α-benzyloxy-5β-chol-11-en-24-oate (110, R = Me, R1 = PhC02, 0.100 g, 0.202 mmol) was treated with potassium hydroxide (0.50 g, 8.91 mmol) in aqueous ethanol (5 %, 20 cm³) and refluxed for 2 h. Evaporation of solvent was followed by addition of water (20 cm³), neutralisation by dropwise addition of aqueous HCl (0.5 M) and extraction with dichloromethane (3 x 20 cm³). Removal of solvent in vacuo yielded the crude acid (0.080 g) which was then dissolved in THF (25 cm³) and treated with a solution of diazomethane in diethyl ether. Evaporation and chromatography (silica, 3 : 1 light petrol / ethyl acetate) yielded the title compound (64 mg, 81 %) as a colourless gum.

IR (CH2Cl2) νmax (cm⁻¹) 2954 (CH), 1732 (C24 C=O); ¹H NMR (250 MHz, CDCl3) δ 0.72 (s, CH3-18, 3H), 0.88 (s, CH3-19, 3H), 1.00 - 1.02 (d, J = 6 Hz, CH3-21, 3H), 3.62 (m, CH-3β, 1H), 3.66 (s, OMe, 3H), 5.42 - 5.43 (dd, J = 10 and 1 Hz, CH-12, 1H), 6.09 - 6.10 (dd, J = 10 and 3 Hz, CH-11, 1H); ¹³C NMR (62.9 MHz, CDCl3) 16.37 (C-18), 18.21 (C-21), 22.90 (C-15), 23.82 (C-19), 25.43 (C-16), 28.00 (C-7), 28.34 (C-6), 30.39 (C-2), 30.79 (C-23), 30.92
(C-22), 34.31 (C-8), 34.89 (C-10), 35.01 (C-1), 35.26 (C-20), 37.00 (C-4), 41.06 (C-9), 43.06 (C-5), 44.91 (C-13), 51.46 (OMe), 51.76 (C-17), 53.55 (C-14), 71.62 (C-3), 125.46 (C-12), 138.63 (C-11), 174.64 (C-24). [Found MH+ 387.2899 (35 %), 404 (100, M+NH4) C25H39O3 Calc. for MH, 387.2899].

Methyl 3-oxo-5β-chol-11-en-24-oate (122, R = Me).

(a) To a rapidly stirred solution of sodium dichromate (42 mg, 0.142 mmol) in distilled water (1 cm³) was added dropwise conc. sulphuric acid (0.1 cm³, 98%). The resulting chromic acid was added to methyl 3α-hydroxy-5β-chol-11-en-24-oate (110, R = Me, R'O = H, 0.05 g, 0.129 mmol) in diethyl ether (10 cm³) at 0 °C and then stirred for 90 minutes. The two-phase mixture was separated, the aqueous layer extracted with diethyl ether (2 x 25 cm³), dried over MgSO₄ and the solvent removed in vacuo to yield a colourless gum. Recrystallisation from acetone yielded the title compound as pale yellow needles (36 mg, 72%).

(b) To methyl 12α-mesyloxy-3-oxo-5β-cholan-24-oate (121, 1.73 g, 3.59 mmol) was heated with potassium acetate (0.70 g, 7.20 mmol) in 1,3-dimethyl-3,4,5,6-tetrahydro-2-pyrimidone (DMPU, 17 cm³) at 140 °C for 4 h. After cooling, the reaction mixture was diluted with dichloromethane (100 cm³) washed with water (50 cm³) and 2 M hydrochloric acid (50 cm³) and dried over MgSO₄. Removal of solvent in vacuo and purification using flash chromatography (silica gel, 4 : 1 light petrol / ethyl acetate) afforded the title compound (1.14 g, 83%).

Mpt. 125 - 126 °C [lit.° mpt. 123 - 124 °C]; IR (CH₂Cl₂ film) νmax (cm⁻¹) 1736 (C₂₄ C=O), 1710 (C₃ C=O); ¹H NMR (400 MHz, CDCl₃) δ 0.76 (s, CH₃-18,
Methyl 12a-hydroxy-3-oxo-5β-cholan-24-oate (120, R = Me).

To a solution of methyl deoxycholate (98, R = OMe, 2.00 g, 4.93 mmol) in toluene (40 cm³) was added cyclohexanone (10 cm³) and aluminium tert-butoxide (1.60 g, 0.65 mmol) and the mixture heated under reflux for 2 h. The cooled reaction mixture was diluted with diethyl ether (100 cm³), then washed with 0.5 M sulphuric acid (2 x 50 cm³) and saturated sodium chloride (100 cm³), dried over MgSO₄ and the solvent removed in vacuo. Purification using flash chromatography (silica, 3 : 7 ethyl acetate / petrol) gave the title compound (1.29 g, 65 %) as colourless needles.

Mpt. 140 - 143 °C [lit.²⁰⁰ Mpt. 140 - 142 °C]; IR (CH₂Cl₂) v_max (cm⁻¹) 3503 (OH), 2940 (CH Aliphatic), 1739 (C=C O), 1713 (C=C O); ¹H NMR (400 MHz, CDCl₃) δ 0.72 (s, CH₃-18, 3H), 0.98 - 0.99 (d, J = 6 Hz, CH₃-21, 3H), 1.01 (s, CH₃-19, 3H), 3.66 (s, OMe, 3H), 4.04 (s, CH-12β, 1H); ¹³C NMR (100MHz, CDCl₃) 12.77 (C-18), 17.73 (C-21), 22.41 (C-19), 23.57 (C-15), 25.48 (C-16), 26.55 (C-7), 27.43 (C-6), 28.96 (C-11), 30.87 (C-23), 31.06 (C-
22), 33.85 (C-9), 34.40 (C-10), 35.06 (C-20), 35.72 (C-8), 36.87 (C-2), 37.11 (C-1), 42.32 (C-4), 44.28 (C-5), 46.57 (C-13), 47.41 (C-17), 48.14 (C-14), 51.53 (OMe), 72.96 (C-12), 174.67 (C-24), 213.36 (C-3). [Found 404.2929 (7%), 386 (8, -H2O), 355 (8), 271 (75)
Calc. for C25H40O4 M, 404.2926].

Methyl 12α-mesyloxy-3-oxo-5β-cholan-24-oate (110, R = Me).²⁷²,²⁷³

To a solution of methyl 12α-hydroxy-3-oxo-5β-cholan-24-oate (120, R = Me, 1.23 g, 3.06 mmol) in pyridine (12 cm³) at 0 °C methane sulphonyl chloride (1.6 cm³, 21.31 mmol) was added dropwise. The resultant orange solution was stirred for 15 h, warming gradually to room temperature, then diluted with dichloromethane (100 cm³), washed successively with copper sulphate solution (2 x 50 cm³), 0.02 M hydrochloric acid (50 cm³) and saturated aqueous sodium chloride (100 cm³). After drying over MgSO₄ and removal of solvent in vacuo, the crude mixture was purified using flash chromatography (silica, 3 : 7 ethyl acetate / petrol) to yield the title compound as a colourless solid (1.23 g, 83%).

Mpt. 64 - 66 °C; IR (CH₂Cl₂) ν max (cm⁻¹) 1732 (C=O), 1709 (C=O); ¹H NMR (250 MHz, CDCl₃) δ 0.80 (s, CH₃-18, 3H), 0.97 - 1.00 (d, J = 6 Hz, CH₃-21, 3H), 1.01 (s, CH₃-19, 3H), 3.06 (s, SCH₃, 3H), 3.66 (s, OMe, 3H), 5.14 (s, C12β-H, 3H); ¹³C NMR (62.9 MHz, CDCl₃) 12.77 (C-18), 17.77 (C-21), 22.48 (C-19), 23.44 (C-15), 25.30 (C-16), 26.58 (C-7), 27.44 (C-6), 27.53 (C-11), 30.86 (C-23), 30.95 (C-22), 34.09 (C-9), 34.51 (C-10), 35.16 (C-20), 35.48 (C-8), 36.83 (C-2), 36.93 (C-1), 39.39 (SCH₃), 42.35 (C-4), 44.25 (C-5), 46.02 (C-13), 47.30 (C-17), 48.46 (C-14), 51.55 (OMe), 76.75 (C-12), 174.46 (C-24).
Diphenyl diselenide (0.01 g, 0.03 mmol) and meta-iodoxybenzoic acid (0.025 g, 0.10 mmol) were heated in toluene (2 cm$^3$) for 15 minutes, then a solution of methyl 3-oxo-5β-chol-11-en-24-oate (122, $R = \text{Me}$, 0.012 g, 0.03 mmol) in toluene (1 cm$^3$) was added and the mixture refluxed for 36 h. Hydrogen peroxide solution (30 cm$^3$, 30%) was added to the cooled reaction mixture and then stirred overnight. The reaction mixture was diluted with ethyl acetate (10 cm$^3$), then washed with saturated aqueous sodium hydrogen carbonate (3 x 5 cm$^3$), dried over MgSO$_4$ and the solvent removed in vacuo. Purification using flash chromatography (silica, 1:3 ethyl acetate/petrol) afforded the title compound as prisms (8 mg, 67%).

M.pt. 108 - 109 °C; IR (CH$_2$Cl$_2$) $\nu_{\text{max}}$ (cm$^{-1}$) 1737 (C24 C=O), 1674 (C3 C=O); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.82 (s, CH$_3$-18, 3H), 1.01 - 1.03 (d, $J = 6$ Hz, CH$_3$-21, 3H), 1.20 (s, CH$_3$-19, 3H), 3.67 (s, OMe, 3H), 5.54 - 5.57 (dd, $J = 10$ and 2 Hz, CH-12, 1H), 6.11 - 6.12 (d, $J = 1$ Hz, CH-4, 1H), 6.26 (dd, $J = 10$ and 3 Hz, CH-11, 1H), 6.27 (dd, $J = 10$ and 1Hz, CH-2, 1H), 7.12 - 7.15 (d, $J = 10$ Hz, CH-1); $^{13}$C NMR (100 MHz, CDCl$_3$) 16.6 (C-18), 18.3 (C-21), 19.4 (C-19), 23.2 (C-15), 28.2 (C-16), 30.8 (C-23), 31.0 (C-22), 32.1 (C-7), 33.6 (C-6), 34.0 (C-8), 35.9 (C-20), 43.6 (C-10), 43.5 (C-13), 51.5 (OMe), 52.0 (C-14), 52.4 (C-17), 54.1 (C-9), 123.6 (C-12), 125.1 (C-4), 127.7 (C-2), 141.0 (C-11), 154.8 (C-1), 168.0 (C-5), 174.5 (C-24), 186.3 (C-3). [Found MH$^+$ 383.2586 (100%), 263 (9) C$_{25}$H$_{35}$O$_3$ requires MH, 383.2586].
Methyl 3-oxo-5β-chola-1,11-dien-24-oate (203), Methyl 3-oxo-5β-chola-4,11-dien-24-oate (202) and Methyl 3-oxo-5β-chola-1,4,11-trien-24-oate (227).

Benzene selenillic anhydride (38 mg, 0.11 mmol) and meta-iodoxybenzoic acid (0.246 g, 1.14 mmol) were heated in anhydrous chlorobenzene (20 cm³) for 15 minutes, then methyl 3-oxo-5β-chol-11-en-24-oate (122, R = Me, 0.22 g, 0.60 mmol) was added and the mixture heated to 120 °C until no starting material remained by TLC. The reaction mixture was allowed to cool, diluted with 50 cm³ ethyl acetate, then washed with aqueous sodium hydrogen carbonate (5 %, 2 x 50 cm³) and saturated sodium chloride (50 cm³), dried over MgSO₄ and the solvent removed in vacuo. Purification using flash chromatography (silica, 1 : 4 ethyl acetate / light petrol) afforded the three title compounds.

(203) Methyl 3-oxo-5β-chola-1,11-dien-24-oate (12 mg, 5 %) as a colourless gum. IR (CDCl₃) νmax (cm⁻¹) 2949 (CH Aliphatic), 1735 (C=O), 1682 (C=O); ¹H NMR (400 MHz, CDCl₃) δ 0.77 (s, CH₃-18, 3H), 1.00 - 1.01 (d, J = 6 Hz, CH₃-21, 3H), 1.13 (s, CH₃-19, 3H), 3.67 (s, OMe, 3H), 5.35 - 5.38 (dd, J = 10 and 2 Hz, CH-12, 1H), 5.93 - 5.96 (d, J = 10 Hz, CH-2, 1H), 6.17 - 6.19 (dd, J = 10 and 3 Hz, CH-11, 1H), 6.85 - 6.88 (d, J = 10 Hz, CH-1, 1H); ¹³C NMR (100 MHz, CDCl₃) 16.59 (C-18), 18.32 (C-21), 21.05 (C-19), 23.01 (C-15), 25.20 (C-16), 27.53 (C-7), 28.20 (C-6), 30.85 (C-23), 31.02 (C-22), 33.73 (C-8), 35.93 (C-20), 38.61 (C-10), 39.66 (C-4), 40.13 (C-5), 44.97 (C-13), 48.99 (C-9), 51.50 (OMe), 51.87 (C-14), 52.67 (C-17), 124.88 (C-12), 127.20 (C-2), 139.97 (C-11), 159.99 (C-1), 174.59 (C-24), 200.47 (C-3).
[Found $\text{M}^+ 384.2664$ (33 %), 369 (21), 269 (100) requires $\text{C}_{25}\text{H}_{36}\text{O}_3\text{M}$, 384.2664].

(202) Methyl 3-oxo-5β-chola-4,11-dien-24-oate (46 mg, 20 %) as a colourless gum. IR (CDCl$_3$) $\nu_{\text{max}}$ (cm$^{-1}$) 2950 (C-H Aliphatic), 1736 (C=C), 1674 (C=C=O); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.79 (s, CH$_3$-18, 3H), 1.01 - 1.03 (d, $J = 6$ Hz, CH$_3$-21, 3H), 1.14 (s, CH$_3$-19, 3H), 3.67 (s, OMe, 3H), 5.41 - 5.44 (dd, $J = 10$ and 2 Hz, CH-12, 1H), 5.75 (s, CH=4, 1H), 6.21 - 6.24 (dd, $J = 10$ and 3 Hz, CH-11, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) 16.6 (C-18), 17.5 (C-19), 18.3 (C-21), 23.0 (C-15), 26.3 (C-16), 30.7 (C-7), 30.9 (C-23), 31.0 (C-22), 33.4 (C-6), 33.8 (C-2), 34.0 (C-8), 35.2 (C-1), 35.9 (C-20), 38.5 (C-10), 44.8 (C-13), 51.5 (OMe), 51.8 (C-14), 52.8 (C-17), 56.1 (C-9), 123.7 (C-12), 125.0 (C-4), 140.1 (C-11), 170.1 (C-5), 174.5 (C-24), 199.3 (C-3). [Found $\text{M}^+ 384.2665$ (25 %), 369 (14), 269 (100), 263 (45) Calc. for $\text{C}_{25}\text{H}_{36}\text{O}_3\text{M}$ 384.2664].

(227) Methyl 3-oxo-5β-chola-1,4,11-trien-24-oate (91 mg, 40 %)

Data as earlier.

Methyl 11α,12α-epoxy-3-oxo-5β-chola-1,4-dien-24-oate (228).

![Diagram of Methyl 11α,12α-epoxy-3-oxo-5β-chola-1,4-dien-24-oate](image)

(a) To methyl 3-oxo-5β-chola-1,4,11-trien-24-oate (123, $R = \text{Me}$, 76 mg, 0.20 mmol), acetonitrile (7.5 cm$^3$), 2,2,2-trifluoroacetophenone (0.17 g, 0.96 mmol) and (EDTA)$\text{Na}_2$ solution ($4 \times 10^{-4}$ M, 5 cm$^3$), was added a solid mixture of Oxone® (0.59 g, 0.96 mmol) and sodium hydrogen carbonate
(0.33g, 3.10 mmol) over 30 minutes. This solution was stirred vigorously at room temperature for 24 h. After such time the reaction mixture was worked up by extraction with ethyl acetate (2 x 25 cm³), washed with saturated sodium hydrogen carbonate solution (50 cm³), dried over Na₂SO₄ and removal of solvent in vacuo to give the title compound as colourless prisms (76 mg, 96 %).

(b)²⁷²,²⁷³ To a solution of methyl 3-oxo-5β-chola-1,4,11-trien-24-oate (123, R = Me, 0.03 g, 0.08 mmol) in dichloromethane (5 cm³) at 0 °C was added meta-chloroperbenzoic acid (0.03 g, 80 % m-CPBA, 0.16 mmol) and sodium hydrogen carbonate (13 mg, 0.16 mmol). The resulting mixture was stirred for 16 h, then diluted with dichloromethane (20 cm³), washed with 5% sodium hydrogen carbonate solution (50 cm³), dried over MgSO₄ and the solvent removed in vacuo. Purification using flash chromatography (silica, 5 : 7 ethyl acetate / petrol) afforded the title compound (25 mg, 82%).

Mpt. 110 - 111 °C (from CH₂Cl₂); IR (CH₂Cl₂) νmax (cm⁻¹) 1732 (C24 C=O), 1664 (C3 C=O), 1625 (C=C); ¹H NMR (400 MHz, CDCl₃) δ 0.87 (s, CH₃-18, 3H), 1.05 (d, J = 6 Hz, CH₃-21, 3H), 1.31 (s, CH₃-19, 3H), 3.18 (d, J = 4 Hz, CH-12, 1H), 3.23 (d, J = 4 Hz, CH-11, 1H), 3.67 (3H, s, OMe), 6.10 (b s, CH-4, 1H), 6.33 (dd, J = 10 and 2 Hz, C2-H, 1H), 7.25 (d, J = 10 Hz, C1-H, 1H); ¹³C NMR (100 MHz, CDCl₃) 12.0 (C-18), 18.3 (C-21), 20.0 (C-19), 22.5 (C-15), 27.1 (C-16), 30.6 (C-7), 31.0 (C-23), 31.5 (C-22), 32.4 (C-8), 33.4 (C-6), 35.1 (C-20), 42.5 (C-10), 43.7 (C-13), 45.1 (C-9), 50.3 (C-17), 50.8 (C-14), 51.5 (OMe), 52.7 (C-11), 60.7 (C-12), 124.9 (C-4), 128.4 (C-2), 153.4 (C-1), 166.6 (C-5), 174.5 (C-24), 186.0 (C-3). [Found MH⁺ 399.2535 (92 %), 316 (12), 214 (100) C₂₅H₅₅O₄ requires MH, 399.2535].
Methyl 11α,12α-epoxy-3-oxo-5β-chola-4-en-24-oate (204).

To methyl 3-oxo-5β-chola-4,11-dien-24-oate (202, 77 mg, 0.20 mmol), acetonitrile (7.5 cm$^3$), 2,2,2-trifluoroacetophenone (0.17 g, 0.96 mmol) and (EDTA)Na$_2$ solution (4 x 10$^{-4}$ M, 5 cm$^3$), was added a solid mixture of Oxone® (0.59 g, 0.96 mmol) and sodium hydrogen carbonate (0.33 g, 3.10 mmol) over 30 minutes. This solution was stirred vigorously at room temperature for 24 h. After such time the reaction mixture was worked up by extraction with ethyl acetate (2 x 25 cm$^3$), washed with saturated sodium hydrogen carbonate solution (50 cm$^3$), dried over Na$_2$SO$_4$ and removal of solvent in vacuo to give the title compound as colourless microcrystalline solid (77 mg, 96 %).

Mpt. 150 - 153 °C [lit. 308 mpt. 152 - 155 °C]; IR (CH$_2$Cl$_2$) $\nu_{max}$ (cm$^{-1}$) 2951 (CH Aliphatic), 1736 (C24 C=O), 1673 (C3 C=O); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.83 (s, CH$_3$-18, 3H), 1.05-1.06 (d, J = 6 Hz, CH$_3$-21, 3H), 1.25 (s, CH$_3$-19, 3H), 2.99 - 3.00 (d, J = 4 Hz, CH-12, 1H), 3.20 - 3.21 (d, J = 4 Hz, CH-11, 1H), 3.67 (s, OMe, 3H), 5.76 (s, CH-4, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) 11.88 (C-18), 17.92 (C-21), 18.27 (C-19), 22.33 (C-15), 27.14 (C-16), 30.40 (C-7), 30.62 (C-23), 30.62 (C-22), 30.92 (C-1), 32.45 (C-8), 33.23 (C-6), 33.51 (C-2), 34.83 (C-17), 35.05 (C-20), 38.27 (C-10), 43.35 (C-14), 45.18 (C-9), 50.23 (OMe), 51.55 (C-13), 52.65 (C-12), 60.57 (C-11), 124.88 (C-4), 168.98 (C-5), 174.56 (C-24), 199.11 (C-3). [Found M$^+$ 400.2612 (25 %), 382 (15, M-H$_2$O), 368 (10) 285 (35) Calc. for C$_{25}$H$_{36}$O$_4$ M, 400.2613].
Methyl 11α,12α-epoxy-3-oxo-5β-cholan-24-oate (205).

To methyl 3-oxo-5β-chol-11,12-en-24-oate (122, R = Me, 77 mg, 0.20 mmol), acetonitrile (7.5 cm³), 2,2,2-trifluoroacetophenone (0.17 g, 0.96 mmol) and (EDTA)Na₂ solution (4 x 10⁻⁴ M, 5 cm³), was added a solid mixture of Oxone® (0.59 g, 0.96 mmol) and sodium hydrogen carbonate (0.33 g, 3.10 mmol) over 30 minutes. This solution was stirred vigorously at room temperature for 24 h. After such time the reaction mixture was worked up by extraction with ethyl acetate (2 x 25 cm³), washed with saturated sodium hydrogen carbonate solution (50 cm³), dried over Na₂SO₄ and removal of solvent in vacuo to give the title compound as a colourless gum (77 mg, 96 %).

IR (CH₂Cl₂) νmax (cm⁻¹) 1736 (C₂₄C=O), 1673 (C₃C=O); ¹H NMR (400 MHz, CDCl₃) δ 0.81 (s, CH₃-18, 3H), 1.04 - 1.06 (d, J = 6 Hz, CH₃-21, 3H), 1.09 (s, CH₃-19, 3H), 2.97 - 2.98 (d, J = 4 Hz, CH-12, 1H), 3.17 - 3.18 (d, J = 4 Hz, CH-11, 1H), 3.67 (3H, s, OMe); ¹³C NMR (100MHz, CDCl₃) 11.95 (C-18), 18.29 (C-21), 22.30 (C-19), 23.28 (C-15), 24.90 (C-16), 27.22 (C-1), 27.38 (C-6), 29.69 (C-22), 31.06 (C-23), 32.48 (C-4), 35.03 (C-10), 35.06 (C-8), 36.48 (C-1), 36.98 (C-20), 41.88 (C-5), 42.68 (C-9), 43.37 (C-13), 43.54 (C-17), 46.27 (C-14), 50.39 (C-13), 51.51 (OMe), 53.12 (C-12), 60.77 (C-11), 174.55 (C-24), 212.48 (C-3). [Found MNH₄⁺ 420.3114 (100 %), 404 (15), 385 (7) C₂₅H₄₄O₄N requires MNH₄, 420.3114].
Methyl 3,12-dioxo-deoxychol-24-ate (132, R = Me).

To a rapidly stirred solution of sodium dichromate dihydrate (0.76 g, 2.58 mmol) in distilled water (4 cm$^3$) was added dropwise conc. sulphuric acid (1 cm$^3$, 98 %). The resulting chromic acid was added to methyl deoxycholate (98, R = Me, 1.00 g, 2.58 mmol) in diethyl ether (50 cm$^3$) at 0 °C and then stirred for 90 minutes. The two-phase mixture was separated, the aqueous layer extracted with diethyl ether (2 x 25 cm$^3$), dried over MgSO$_4$ and the solvent removed in vacuo to yield a colourless gum. Recrystallisation from MeOH yielded the title compound as white prisms (0.62 g, 60 %).

Mpt. 133 - 134 °C [lit.$^{304}$ mpt. 131 - 133 °C]; IR (CH$_2$Cl$_2$) $\nu_{\text{max}}$ (cm$^{-1}$) 2934 (CH Aliphatic), 1737 (C24 C=O), 1709 (C3 + C12 C=O); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.85 - 0.87 (d, $J = 6$ Hz, CH$_3$-21, 3H), 1.11 (s, CH$_3$-18, 3H), 1.33 (s, CH$_3$-19, 3H), 3.66 (s, OMe, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) 11.73 (C-18), 18.60 (C-21), 22.14 (C-19), 24.32 (C-15), 25.48 (C-6), 26.62 (C-22), 27.49 (C-23), 30.52 (C-16), 31.30 (C-7), 35.46 (C-20), 35.62 (C-5), 36.82 (C-2), 36.91 (C-11), 38.37 (C-1), 42.13 (C-4), 42.17 (C-10), 43.72 (C-9), 44.28 (C-14), 46.55 (C-17), 51.55 (OMe), 57.58 (C-13), 58.54 (C-8), 174.57 (C-24), 211.95 (C-3), 214.01 (C-12). [Found M$^+$ 402.2768 (100 %), 329 (13), 287 (12), 247 (96) Calc. for C$_{25}$H$_{38}$O$_4$ M, 402.2769].
Methyl 3,12-dioxo-5β-chol-1-en-24-oate (211) and Methyl 3,12-dioxo-5β-chol-4-en-24-oate (210).

A mixture of methyl 3,12-dioxo-5β-cholan-24-oate (132, R = Me, 0.10 g, 0.25 mmol), meta-iodoxybenzoic acid (0.35 g, 1.25 mmol) and diphenyl diselenide (31 mg, 0.10 mmol) in toluene (15 cm³) was refluxed under a nitrogen atmosphere for 18 h. After cooling, the reaction mixture was thoroughly extracted with saturated NaHCO₃ (3 x 25 cm³) and the organic phase was washed with water (2 x 25 cm³) and dried over Na₂SO₄. Removal of the solvent in vacuo and purification using flash chromatography (silica, 4 : 1 dichloromethane / diethyl ether) gave (210, 69 mg, 70%) and (211, 3 mg, 3%).

(211) Mpt. 88 - 90 °C; IR (CDCl₃) νmax (cm⁻¹) 1736 (C₂₄ C=O), 1706 (C₁₂ C=O), 1681 (C₃ C=O); ¹H NMR (400 MHz, CDCl₃) δ 0.84 - 0.85 (d, J = 6 Hz, CH₃-21, 3H), 1.03 (s, CH₃-18, 3H), 1.16 (s, CH₃-19, 3H), 3.67 (s, OMe, 3H), 5.92 - 5.94 (d, J = 10 Hz, CH-2), 6.66 - 6.69 (d, J = 10 Hz, CH-1, 1H); ¹³C NMR (100 MHz, CDCl₃) 11.71 (C-18), 18.55 (C-21), 20.39 (C-19), 24.33 (C-15), 25.64 (C-6), 26.43 (C-22), 27.30 (C-23), 30.40 (C-16), 31.24 (C-7), 34.98 (C-20), 35.55 (C-5), 38.80 (C-11), 38.92 (C-4), 38.99 (C-10), 40.43 (C-9), 46.46 (C-14), 48.72 (C-17), 51.55 (OMe), 57.41 (C-13), 57.73 (C-8), 127.71 (C-2), 158.98 (C-1), 174.64 (C-24), 200.12 (C-3), 213.48 (C-12.

[Found M⁺ 400.2613 (60%), 327 (20), 285 (20) Calc. for C₂₅H₃₆O₄ M, 400.2621].
(210) Mpt. 138 - 141 °C [lit.\(^{306}\) mpt. 142-143 °C]; IR (CH\(_2\)Cl\(_2\)) \(\nu_{\text{max}}\) (cm\(^{-1}\))

1735 (C24 C=O), 1706 (C12 C=O), 1682 (C3 C=O); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.85 - 0.87 (d, \(J = 6\) Hz, CH\(_3\)-21, 3H), 1.09 (s, CH\(_3\)-18, 3H), 1.28 (s, CH\(_3\)-19, 3H), 3.67 (s, OMe, 3H), 5.78 (s, CH-4, 1H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) 11.68 (C-18), 16.92 (C-21), 18.59 (C-19), 24.35 (C-15), 27.37 (C-6), 30.51 (C-22), 31.02 (C-23), 31.27 (C-16), 32.56 (C-7), 33.72 (C-2), 35.35 (C-20), 35.44 (C-11), 35.58 (C-9), 37.93 (C-1), 38.96 (C-10), 46.46 (C-14), 51.40 (OMe), 56.21 (C-17), 56.96 (C-13), 57.39 (C-8), 124.69 (C-4), 168.67 (C-5), 174.47 (C-24), 198.68 (C-3), 213.17 (C-12). [Found M\(^{+}\) 400.2618 (100 %), 327 (22), 245 (85) Calc. for C\(_{25}\)H\(_{36}\)O. M, 400.2621].

**Methyl 3,12-dioxo-5β-chola-1,4-dien-24-oate (226).**

(a) A mixture of methyl 3,12-dioxo-5β-cholan-24-oate (132, R = Me, 0.10 g, 0.25 mmol) and DDQ (0.164 g, 0.72 mmol) in anhydrous benzene (10 cm\(^3\)) was refluxed for 24 h. The suspension was cooled, filtered and the filtrate washed several times with 1 % potassium hydroxide solution (3 x 25 cm\(^3\)), then with water (25 cm\(^3\)). The solution was dried over MgSO\(_4\), concentrated *in vacuo* and purification using flash chromatography (silica, 4 : 1 light petrol / ethyl acetate) to give the title compound as prisms (39 mg, 40 %).

(b) A mixture of methyl 3,12-dioxo-5β-cholan-24-oate (132, R = Me, 0.10 g, 0.25 mmol) and selenium dioxide (0.58 g, 0.53 mmol) in t-butanol (15 cm\(^3\)) with acetic acid (0.1 cm\(^3\)) were refluxed for 18 h. At the end of this time the reaction solution was yellow and black selenium had deposited at the bottom of the flask. The solution was filtered, concentrated *in vacuo* and purification using flash chromatography (silica, 4 : 1 light petrol / ethyl acetate) to give the title compound as a colourless solid (32 mg, 32 %).
(c) A mixture of methyl 3,12-dioxo-5β-cholan-24-oate (132, R = Me, 0.20 g, 0.5 mmol), meta-iodoxybenzoic acid (0.70 g, 2.50 mmol) and diphenyl diselenide (0.031 g, 0.10 mmol) in toluene (20 cm³) was refluxed under a nitrogen atmosphere. After 18 h a further portion of meta-iodoxybenzoic acid (0.375 g, 1.25 mmol) was added and heating was continued for a further 18 h. After cooling, the reaction mixture was thoroughly extracted with saturated NaHCO₃ (3 x 50 cm³) and the organic phase was washed with water (2 x 50 cm³) and dried over Na₂SO₄. Removal of the solvent in vacuo and purification using flash chromatography (silica, 2 : 1 dichloromethane / diethyl ether) gave the title compound (0.152 g, 76 %).

Mpt. 135 - 137 °C (from CH₂Cl₂) [lit.²⁸⁵ mpt. 136 - 137 °C]; IR (CH₂Cl₂) νₓmax (cm⁻¹) 1736 (C24 C=O), 1707 (C12 C=O), 1664 (C3 C=O); ¹H NMR (400 MHz, CDCl₃) δ 0.86 - 0.88 (d, J = 6 Hz, CH₃-21, 3H), 1.11 (s, CH₃-18, 3H), 1.32 (s, CH₃-19, 3H), 3.66 (s, OMe, 3H), 6.09 (d, J = 2 Hz, CH-4), 6.23 - 6.26 (dd, J = 2 and 10 Hz, CH-2, 1H), 6.85 - 6.88 (d, J = 10 Hz, CH-1, 1H); ¹³C NMR (100 MHz, CDCl₃) 11.67 (C-18), 18.46 (C-21), 18.58 (C-19), 24.52 (C-15), 27.30 (C-16), 30.46 (C-22), 31.24 (C-23), 32.29 (C-6), 32.52 (C-7), 35.28 (C-20), 35.54 (C-14), 39.36 (C-11), 43.45 (C-10), 46.51 (C-17), 51.39 (OMe), 53.81 (C-9), 56.19 (C-8), 56.55 (C-13), 125.06 (C-4), 128.00 (C-2), 153.90 (C-1), 166.83 (C-5), 174.39 (C-24), 185.86 (C-3), 211.99 (C-12). [Found M⁺ 398.2453 (34 %), 277 (28), 245 (39) Calc. for C₂₅H₃₄O₄ M, 398.2457].
Methyl 3,12-dioxo-5\(\beta\)-chola-1,4,9(11)-trien-24-oate (225).

Methyl 3,12-dioxo-5\(\beta\)-cholan-24-oate (132, R = Me, 0.201 g, 0.5 mmol) was refluxed under identical conditions as that for (226, Method (c)) above. Another portion of meta-iodoxybenzoic acid (0.375 g, 1.25 mmol) was then added and heating was continued for a further 36 h. After cooling, the reaction mixture was thoroughly extracted with saturated NaHCO\(_3\) (3 x 50 cm\(^3\)) and the organic phase was washed with water (2 x 50 cm\(^3\)) and dried over Na\(_2\)SO\(_4\). Removal of the solvent in vacuo and purification using flash chromatography (silica, 2 : 1 dichloromethane / diethyl ether) gave the title compound (0.122 g, 62 %).

Mpt. 137 - 139 °C (from CH\(_2\)Cl\(_2\)) [lit.\(^{285}\) mpt. 137 - 139 °C]; IR (CH\(_2\)Cl\(_2\)) \(\nu_{\text{max}}\) (cm\(^{-1}\)) 1735 (C24 C=O), 1686 (C12 C=O), 1666 (C3 C=O); \(^1\)H NMR (400 MHz, CDC\(_3\)) \(\delta\) 1.01 - 1.02 (d, \(J = 6\) Hz, CH\(_3\)-21 & s, CH\(_3\)-18, 6H), 1.55 (s, CH\(_3\)-19, 3H), 3.66 (s, OMe, 3H), 5.74 - 5.75 (d, \(J = 4\) Hz, CH-11, 1H) 6.12 - 6.14 (b s, CH-4, 1H), 6.30 - 6.35 (dd, \(J = 3\) and 16 Hz, CH-2, 1H), 7.10 - 7.14 (d, \(J = 16\) Hz, CH-1, 1H); \(^{13}\)C NMR (100 MHz, CDC\(_3\)) 10.91 (C-18), 19.25 (C-21), 24.33 (C-15), 27.29 (C-19), 27.54 (C-16), 30.54 (C-22), 31.34 (C-23), 31.60 (C-6), 33.04 (C-7), 35.30 (C-20), 38.00 (C-14), 45.74 (C-10), 46.98 (C-17), 51.46 (OMe), 52.59 (C-8), 53.03 (C-13), 123.04 (C-11), 125.06 (C-4), 128.15 (C-2), 151.86 (C-1), 162.60 (C-9), 163.89 (C-5), 174.58 (C-24), 185.53 (C-3), 204.07 (C-12). [Found M\(^+\) 396.2298 (20 %), 241 (100), 223 (13) Calc. for C\(_{25}\)H\(_{32}\)O\(_4\) M, 396.2300].
Methyl 1-hydroxy-4-methyl-12-oxo-5β-chola-1,3,5(10)-trien-24-oate (230).

A solution of methyl 3,12-dioxo-5β-chola-1,4-dien-24-oate (226, 0.2 g, 0.502 mmol) in pyridine (10 cm³) with ‘activated’ zinc dust (2.00 g, 30.60 mmol) and water (0.5 cm³) was refluxed with vigorous stirring for 12 h. After cooling, the mixture was diluted with dichloromethane (50 cm³) and filtered through a short pad of Celite®. The filtrate was washed with 2 M HCl (3 x 50 cm³), saturated NaHCO₃ (50 cm³) and water (50 cm³), dried over Na₂SO₄ and solvent evaporated in vacuo which gave the title compound as a colourless gum (0.18 g, 90%).

IR (CH₂Cl₂) ν_{max} (cm⁻¹) 3266 (OH), 1737 (C24 C=O), 1706 (C12 C=O);

¹H NMR (400 MHz, CDCl₃) δ 0.96 - 0.98 (d, J = 6 Hz, CH₃-21, 3H), 1.09 (s, CH₃-18, 3H), 2.15 (s, CH₂-4, 3H), 3.67 (s, O-CH₃, 3H), 4.13 - 4.18 (dd, J = 4 and 9 Hz, CH-9, 1H), 6.63 - 6.65 (d, J = 8 Hz, CH-2, 1H), 6.86 - 6.88 (d, J = 8 Hz, CH-3, 1H), 8.00 (b, OH, 1H); ¹³C NMR (100 MHz, CDCl₃) 12.26 (C-18), 19.15 (C-21), 19.58 (C-4'), 24.27 (C-15), 26.33 (C-16), 28.02 (C-22), 29.58 (C-23), 31.07 (C-6), 31.81 (C-7), 36.29 (C-20), 39.97 (C-8), 42.27 (C-11), 47.20 (C-14), 47.36 (C-17), 51.88 (OMe), 57.36 (C-9), 58.50 (C-13), 113.35 (C-2), 125.19 (C-4), 127.66 (C-10), 128.62 (C-3), 138.10 (C-5), 154.45 (C-1), 175.09 (C-24), 218.66 (C-12). [Found M⁺ 398.2455 (4 %), 396 (34), 365 (8) C₂₉H₃₄O₄ requires M, 398.2457].

A solution of methyl 3,12-dioxo-5β-chola-1,4,11-trien-24-oate (225, 0.1 g, 0.25 mmol) in pyridine (10 cm³) with ‘activated’ zinc dust (2.00 g, 30.60 mmol) and water (0.5 cm³) was refluxed with vigorous stirring for 12 h. After cooling, the mixture was diluted with dichloromethane (50 cm³) and filtered through a short pad of Celite. The filtrate was washed with 2 M HCl (3 x 50 cm³), saturated NaHCO₃ (50 cm³) and water (50 cm³), dried over Na₂SO₄ and solvent evaporated in vacuo. Recrystallisation from methanol gave the title compound as colourless needles (83 mg, 86%).

Mpt. 165 - 167 °C (from methanol) [lit.²⁸⁵ mpt. 165 - 166 °C]; IR (CH₂Cl₂) ʋ max (cm⁻¹) 3374 (OH), 1731 (C=O), 1654 (C=O); ¹H NMR (400 MHz, CDCl₃)  δ 1.02 (s, CH3-18, 3H), 1.74 - 1.90 (d, J = 6 Hz, CH₃-21, 3H), 3.68 (s, OMe, 3H), 6.25 - 6.26 (d, J = 2 Hz, CH-11, 1H), 6.65 - 6.66 (d, J = 3 Hz, CH-4, 1H), 6.71 - 6.74 (dd, J = 3 and 9 Hz, CH-2, 1H), 7.14 (b, OH, 1H), 7.56 - 7.58 (d, J = 9 Hz, CH-1, 1H); ³¹C NMR (100 MHz, CDCl₃) 19.19 (C-18), 17.76 (C-21), 22.10 (C-16), 25.10 (C-15), 26.29 (C-22), 27.89 (C-23), 28.64 (C-6), 29.63 (C-7), 33.24 (C-20), 37.88 (C-14), 45.62 (C-17), 49.58 (C-13), 50.97 (C-8), 51.07 (OMe), 111.62 (C-4), 112.60 (C-2), 113.55 (C-11), 121.78 (C-10), 125.56 (C-1), 139.40 (C-5), 154.58 (C-3), 156.41 (C-9), 173.05 (C-24), 204.18 (C-12). [Found M⁺ 382.2144 (28 %), 267 (4), 227 (100) Calc. for C₂₄H₃₀O₄ M, 382.2144].
Methyl 3-hydroxy-10-methyl-12-oxo-9,10-seco-chola-1,3,5(10),9(11)-tetraen-24-oate (231).

A solution of methyl 11α,12α-epoxy-3-oxo-5β-chola-1,4-dien-24-oate (228, 0.1 g, 0.25 mmol) in pyridine (10 cm³) with 'activated' zinc dust (2.00 g, 30.60 mmol) and water (0.5 cm³) was refluxed with vigorous stirring for 12 h. After cooling, the mixture was diluted with dichloromethane (50 cm³) and filtered through a short pad of Celite. The filtrate was washed with 2 M HCl (3 x 50 cm³), saturated NaHCO₃ (50 cm³) and water (50 cm³), dried over Na₂SO₄ and solvent evaporated in vacuo which gave the title compound as a colourless gum (96 mg, 96 %).

IR (CH₂Cl₂) νmax (cm⁻¹) 3388 (OH), 1737 (C₂₄C=O), 1682 (C₁₂C=O); ¹H NMR (400 MHz, CDCl₃) δ 1.01 - 1.03 (s, CH₃-18 & d, J = 6 Hz, CH₃-21, 6H), 2.21 (s, CH₃-10, 3H), 3.67 (s, OMe, 3H), 4.95 (b, OH, 1H), 5.86 - 5.89 (dd, J = 3 and 10 Hz, CH-11, 1H), 6.58 - 6.61 (dd, J = 3 and 8 Hz, CH-2, 1H), 6.62 - 6.63 (d, J = 3 Hz, CH-4); 6.66 - 6.69 (dd, J = 2 and 10 Hz, CH-9), 6.98 - 7.00 (d, J = 8 Hz, CH-1); ¹³C NMR (100 MHz, CDCl₃) 10.43 (C-18), 18.39 (C-21), 19.54 (C-19), 24.35 (C-16), 26.79 (C-15), 30.08 (C-22), 30.59 (C-23), 31.50 (C-6), 32.72 (C-7), 35.30 (C-20), 40.03 (C-8), 47.35 (C-14), 51.54 (OMe), 52.49 (C-17), 53.67 (C-13), 112.82 (C-2), 115.50 (C-4), 127.63 (C-10), 128.97 (C-11), 131.21 (C-1), 141.49 (C-5), 149.53 (C-9), 153.84 (C-3), 174.80 (C-24), 205.76 (C-12). [Found M⁺ 398.2455 (4 %), 277 (35), 245 (23) C₂₅H₃₄O₄ requires M, 398.2457].
Methyl 1-acetoxy-4-methyl-12-oxo-5\(\beta\)-chola-1,3,5(10)-trien-24-oate (237).

Acetic anhydride (0.03 g, 0.30 mmol) was added dropwise to a solution of Methyl 1-hydroxy-4-methyl-12-oxo-5\(\beta\)-chola-1,3,5(10)-trien-24-oate (230, 0.01 g, 0.025 mmol) in anhydrous pyridine (5 cm\(^3\)) over 2 h. After stirring for 16 h at room temperature, the solution was then poured over iced water (20 cm\(^3\)) and extracted into CH\(_2\)Cl\(_2\) (3 x 10 cm\(^3\)). The combined extracts were washed with HCl (2M, 20 cm\(^3\)), H\(_2\)O (20 cm\(^3\)), sat. NaHCO\(_3\) (20 cm\(^3\)), dried over MgSO\(_4\) and concentrated in vacuo. Purification using flash chromatography (silica gel, 2 : 1 light petrol / diethyl ether) gave the title compound as a colourless gum (11 mg, 98%).

IR (CH\(_2\)Cl\(_2\)) \(\nu_{\text{max}}\) (cm\(^{-1}\)) 3058 (CH Aromatic), 2949 (CH Aliphatic), 1761 (CH\(_3\)CO-Ar), 1737 (C24 C=O), 1704 (C12 C=O); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.90 - 0.91 (d, \(J = 6\) Hz, CH\(_3\)-21, 3H), 1.02 (s, CH\(_3\)-18, 3H), 2.21 (s, CH\(_3\)-4, 3H), 2.36 (s, CH\(_3\)CO-1', 3H), 3.67 (s, OMe, 3H), 6.77 - 6.79 (d, \(J = 8\) Hz, CH-2, 1H), 7.03 - 7.05 (d, \(J = 8\) Hz, CH-3, 1H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) 11.74 (C-18), 18.68 (CH\(_3\)CO-1') 19.72 (C-21), 21.31 (C-4'), 23.88 (C-15), 25.72 (C-16), 27.54 (C-22), 29.08 (C-23), 30.58 (C-6), 31.30 (C-7), 35.78 (C-20), 39.31 (C-8), 43.25 (C-11), 46.11 (C-14), 46.93 (C-17), 51.50 (OMe), 56.54 (C-9), 57.78 (C-13), 120.17 (C-2), 128.43 (C-3), 130.43 (C-4), 134.63 (C-10), 138.18 (C-5), 147.71 (C-1), 169.63 (CH\(_3\)CO-C1'), 174.65 (C-24), 213.53 (C-12). [Found M\(^+\) 440.2555 (1 %), 380 (78), 283 (38) C\(_{27}\)H\(_{36}\)O\(_5\) requires M, 440.2562].
A solution of methyl 3-hydroxy-12-oxo-19-\textit{nor}-5\beta-chola-1,3,5(10),9(11)-tetraen-24-oate (229, 0.01 g, 0.013 mmol) and platinum oxide (6 mg, 0.026 mmol) in acetic acid (1 cm$^3$) and ethyl acetate (1 cm$^3$) were stirred under nitrogen for 30 minutes. Hydrogen was then applied to the flask by means of a hydrogen filled balloon and the reaction mixture was stirred overnight. The mixture was then poured over iced water (20 cm$^3$) and extracted into ethyl acetate (3 x 10 cm$^3$). The combined extracts were washed with sat. NaHCO$_3$ (20 cm$^3$), HCl (2M, 10 cm$^3$), H$_2$O (10 cm$^3$), dried over MgSO$_4$ and concentrated \textit{in vacuo}. Purification using flash chromatography (silica gel, 4 : 1 light petrol / EtOAc) gave the title compound as a colourless gum (2 mg, 20%).

IR (CDCl$_3$) $\nu_{\text{max}}$ (cm$^{-1}$), 1736 (C24 C=O), 1710 (C12 C=O); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.69 (s, CH$_3$-18, 3H), 0.96 - 0.97 (d, $J = 6$ Hz, CH$_3$-21, 3H), 3.60 (s, OMe, 3H), 4.68 (b, OH, 1H), 6.49 (d, $J = 2$ Hz, CH-4, 1H), 6.49 - 6.56 (dd, $J = 2$ and 8 Hz, CH-4, 1H), 6.93 - 6.99 (d, $J = 8$ Hz, CH-1, 1H).

Acetic anhydride (0.03 g, 0.30 mmol) was added dropwise to a solution of methyl 3-hydroxy-12-oxo-19-nor-5β-chola-1,3,5(10),9(11)-tetaen-24-oate (229, 15 mg, 0.039 mmol) in anhydrous pyridine (5 cm³) over 2 h. After stirring for 16 h at room temperature, the solution was then poured over iced water (20 cm³) and extracted into CH₂C₁₂ (3 x 10 cm³). The combined extracts were washed with HCl (2M, 20 cm³), H₂O (20 cm³), sat. NaHCO₃ (20 cm³), dried over MgSO₄ and concentrated in vacuo. Purification using flash chromatography (silica gel, 2 : 1 light petrol / diethyl ether) gave the title compound as colourless needles (16 mg, 96 %).

Mpt. 162 - 164 °C [lit. 285 mpt. 164 - 165 °C]; IR (CDCl₃) ν_max (cm⁻¹) 3056 (CH Aromatic), 2928 (CH Aliphatic), 1764 (CH₃CO-Ar), 1737 (C₂₄ C=O), 1669 (C₁₂ C=O); ¹H NMR (400 MHz, CDCl₃) δ 1.02 (s, CH₃-18, 3H), 1.06 - 1.07 (d, J = 6 Hz, CH₃-21, 3H), 2.23 (s, CH₃CO, 3H), 3.67 (s, OMe, 3H), 6.33 - 6.34 (d, J = 2 Hz, CH-11, 1H), 6.91 - 6.92 (d, J = 2 Hz, CH-4, 1H), 7.71 - 7.73 (dd, J = 2 and 9 Hz, CH-2, 1H), 7.14 (b. s, OH, 1H), 7.56 - 7.58 (d, J = 9 Hz, CH-1, 1H); ¹³C NMR (100 MHz, CDCl₃) 10.94 (C-18), 19.64 (C-21), 21.14 (CH₃CO-3), 22.70 (C-16), 24.13 (C-15), 28.07 (C-22), 29.76 (C-23), 30.67 (C-6), 31.57 (C-7), 35.33 (C-20), 39.66 (C-14), 47.52 (C-17), 51.49 (OMe), 53.07 (C-13), 53.11 (C-8), 119.98 (C-4), 120.52 (C-2), 122.12 (C-11), 126.96 (C-1), 129.52 (C-10), 140.63 (C-5), 151.75 (C-3), 154.07 (C-9), 169.30 (CH₃CO-3), 174.73 (C-24), 204.18 (C-12). [Found M⁺ 424.2252 (4 %), 382 (18), 269 (47), 227 (100) Calc. for C₂₆H₃₂O₅ M, 424.2250].
References.
   (b) Pearl, L.H.; Taylor, W.R. *Nature* 1987, 328, 482.


19 Humphrey, M.J.; Ringrose, P.S. *Drug Metabolism Reviews* 1986, 17, 283.


26 Dreyer, G.B.; Metcalf, B.W.; Tomaszek, T.A.; Carr, T.J.; Chandler, A.C.;
Hyland, L.; Fakhoury, S.A.; Maggard, V.W.; Moore, M.L.; Strickler, J.E.;

27 Tomasselli, A.G.; Olsen, M.K.; Hui, J.O.; Staples, D.J.; Sawyer, T.K.;

and Biology." Proceeding of the Eleventh American Peptide
Symposium 1990, Rivier, J.E.; Marshall, G.R.; Ed., ESCOM, Leiden,
835.

29 Richards, A.D.; Roberts, R.; Dunn, B.M.; Graves, M.C.; Kay, J. FEBS

30 Moore, M.L.; Bryan, W.M.; Fakhoury, S.A.; Maggaard, V.W.; Huffman,
Strickler, J.E.; Gorniak, J.G.; Debouck, C. Biochemical and Biophysical
Research Communications 1989, 159, 420.

Moore, M.L.; Strickler, J.E.; Debouck, C.; Hyland, L.J.; Matthews, T.J.;

32 Tomasselli, A.G.; Hui, J.O.; Sawyer, T.K.; Staples, D.J.; Bannow, C.;
Reardon, I.M.; Howe, W.J.; DeCamp, D.L.; Craik, C.S.; Heinrikson, R.L.;
J. Biol. Chem. 1990, 265, 14675.

33 Vacca, J.P.; Guare, J.P.; deSolms, S.J.; Sanders, W.M.; Giuliani, E.A.;
Young, S.D.; Darke, P.L.; Zugay, J.; Sigal, I.S.; Scheif, W.A.; Quintero,
1225.

34 Ashorn, P.; McQuade, T.J.; Thaisvirongs, S.; Tomasselli, A.G.; Tarpley,

35 Grobelny, D.; Wondrak, E.M.; Galardy, R.E.; Oroszlan, S. Biochemical
and Biophysical Research Communications 1990, 169, 1111.

Chem. 1990, 33, 1285.


47 Ghosh, A.K.; Thompson, W.J.; McKe, S.P.; Duong, T.T.; Lyle, T.A.

48 Pollard, R.B. *Pharmacotherapy* 1994, 14, 21S-29S.


James, J.C.; Semmelhack, M.F. *Bioorganic and Medicinal Chemistry*

58 Abdell-Meguid, S.S.; Zhao, B.; Murthy, K.H.M.; Winborne, E.; Choi, J.K.;
DesJarlais, R.L.; Minnich, M.D.; Culp, J.S.; Debouck, C.; Tomaszek,

59 Holmes, D.S.; Bethell, R.C.; Cammack, N.; Clemens, I.R.; Kitchin, J.;
McMeekin, P.; Mo, C.L.; Orr, D.C.; Patel, B.; Paternoster, I.L.; Storer, R.;

60 Humber, D.C.; Bamford, M.J.; Bethell, R.C.; Cammack, N.; Cobley, K.;
Evans, D.N.; Gray, N.M.; Hann, M.M.; Orr, D.C.; Saunders, J.; Shenoy,
36, 3120.

61 Olson, G.L.; Bolin, D.R.; Bonner, D.R.; Bos, M.; Cook, C.M.; Fry, D.C.;
Graves, B.J.; Hatada, M.; Hill, D.E.; Kahn, M.; Madison, V.S.; Rusiecki,
1993, 36, 3039.


63 (a) Ondeyke, J.; Hensens, O.D.; Zink, D.; Ball, R.; Lingham, R.B.; Bills,
(b) Hungate, R.W.; Chen, J.L.; Starbuck, K.E.; Macaluso, S.A.; Rubino,

64 Ashok, A.D.; Kokke, W.C.; Cochran, S.; Francis, T.A.; Tomaszek, T.;


Yang, C.P.; Stohbach, J.W.; Turner, S.; McGrath, J.; Bohanon, M.; Lynn,
J.; Mulichak, P.; Spinelli, P.; Hinshaw, R.; Pagano, P.J.; Moon, J.B.;
(b) Prasad, J.V.N.V.; Para, K.S.; Lunney, E.A.; Ortwine, D.F.; Dunbar,


Sui, Z.; DeVoss, J.J.; DeCamp, D.L.; Li, J.; Craik, C.S.; Ortiz de Montellano, P.R. *Synthesis* 1993, 803.


DeClerq, E. *Biochemical Pharmacology* 1994, 47, 155.


96 from ESV, Tripos Associates, St. Louis, USA.


(b) Dayil, B.; Speck, J.; Bagan, E.; Tint, E.; Salen, G. *Steroids* 1980, 39


123 Dane, B. Z. *Phys. Chem.* 1936, **244**, 241.


133 Piancatelli, A; Scettri, A; D’Auria, M. Synthesis 1982, 245.
139 Touster, O. Org. React. 1967, Chpt. 6, 327.
158 Koziara, A. Synthesis 1987, 487.
163 Bergmann, M.; Zervas, L. Ber. 1932, 65, 1192.
172 McKenzie, B.F.; Mattox, V.R.; Kendall, E.C. J. Biol. Chem. 1948, 175,
249.

174 Augustine, R.L. "Organic Reactions in Steroid Chemistry" 1972, 1, Ch. 3, 111, Litten Educational Publishing Inc.
This technique is discussed in the technical bulletin 'Quantitative Analysis of Active Boron Hydrides' which is available upon request from the Aldrich Chemical Company, Inc.


A similar sequence to this was described by W. Kreiser and U. Kock at the 14th Conference on Isoprenoids in Táber, 1991.


218 from Aldrich Chemicals.
226 Barllett, P.D. Rec. Chem. Progr. 1950, 2, 47.
240 Boehlow, T.R.; Buxton, P.C., Grocock, E.L.; Marples, B.A.; Waddington,
Reactions were ran in 1.5:1 CH$_3$CN/EDTA solution (4 x 10$^{-4}$M), 1 equiv. of alkene, 5 equivs. of 2,2,2-trifluoroacetophenone, 5 equivs. Oxone$^\circledast$, 15.5 equivs. NaHCO$_3$ at room temperature for 24 h with vigorous stirring.
67, 1425.

281 By $^1$H NMR.


287 For a review see Kirk, D.N.; Hartsham, M.P. "Steroid Reaction Mechanisms" 1968, Elsevier, Amsterdam, 277


Dane, B. Z. Physiol. Ch. 1936, 244, 241


